Tiered laboratory analyses for common infections to characterize febrile morbidity not related to malaria in Sierra Leone

Thesis submitted in accordance with the requirements of the Liverpool School of Tropical Medicine for the degree of Doctor in Philosophy.

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ABSTRACT

In tropical Africa, fever is commonly associated with malaria. However, there are many other illnesses presenting with fever. Non-malaria febrile illnesses (NMFIs) may be attributable to multiple etiologic agents including viral, bacterial and parasitic infections in malaria-endemic resource-poor countries. NMFIs pose challenges to peripheral health systems such that they are clinically under-diagnosed while malaria remains over-diagnosed. Misdiagnoses of a febrile condition may lead to wrong prescription that delays treatment and increases expenditure on health-care and also leads to increased morbidity and mortality. In Sierra Leone, dealing with infections other than malaria remain a serious problem, starting from diagnosis to providing care. Several factors make it difficult to test and treat for NMFIs. Fewer febrile people report their fevers to healthcare centers and there are fewer resources generally which include: fewer laboratories, insufficiently trained laboratory technicians, inadequate standardized infrastructure and unsuitable equipment, epileptic power supplies as well as poor cold-chain storage conditions for reagents among others.

The primary goal of this Ph.D. study was to investigate the prevalence/incidence of NMFIs in Bo, Sierra Leone, using a tiered laboratory analyzes method. The specific objectives were to: investigate the types and etiology of non-malarial febrile illnesses in Bo, Sierra Leone; determine the prevalence/incidence of non-malarial pathogens causing febrile illnesses, and investigate the distribution of NMFIs.

The study started with a baseline and syndromic survey of all households in the study community (n=882 households with 5410 persons). A total cohort of 1403 persons was recruited and followed for a period of one year. After obtaining informed-consent, bio-samples were obtained from febrile subjects and used for laboratory analyses involving three tiers.

The first tier (T1) included the use of rapid, lateral flow assays (RLFAs). T1 tests were: chikungunya, malaria, typhoid fever, syphilis, HIV, hepatitis A, B and C, dengue fever, leptospirosis, influenza A and B, RSV and *Streptococcus aureus*. Subsequent tests at Tier 2 included singleplex and multiplex PCR and bacterial culture; with resequencing pathogen microarray at Tier 3.

From the initial survey 882 households with 5410 individuals and 76.6% reported having malaria in a month prior to the study. About 1402 (25.9%) of persons in participating households were reported to have had a fever within the past six months. The rate of fever reported differed by age group and sex, with young children having the highest rate ($p<0.001$) and females reporting more fevers than males ($p<0.001$).

Viral infections detected included; 46% chikungunya (95%CI 43.5-48.7), 24.2 human rhino virus/enterovirus (95%CI: 17.4-32.6), 19.2% corona virus (95%CI: 13.1-27.1), 9.7% HIV (95%CI: 8.2-11.4), 8.5% hepatitis B(HbSAg) (95%CI: 7.1-10.1), 8.7%HAV(IgG)(95%CI:7.3-10.3), 8.3% influenza B (95%CI:4.6-14.7), 5% adenovirus(95%CI: 2.1-11.0), 4.7% hepatitis C(95%CI: 3.7-5.9), 2.8% dengue fever (95%CI: 2.0-3.8), 1.7% parainfluenza virus and 1.7% influenza A(H1N1) (95%CI: 0.5-5.9), 0.8% cytomegalovirus (95%CI: 0.04-5.2) and 0.2 % human coxsackie virus A24 and A22(95%CI: 0.07-0.6).

Bacterial infections detected included: 16.9% of *Escherichia. coli* (95%CI: 11.6-23.9); 12.6% of *Klebsiella pneumonia* (95%CI: 8.2-19.2); 12% of *Citrobacter freundii* (95%CI: 7.6-18.3); 8.5% of *Enterobacter cloacae (*95%*CI:* 4.9-14.2), 7.5% *Haemophilus influenzae* (95%CI: 3.7-14.2), *5%Chlamydophila pneumonia (95%CI:* 2.9-11.6), 4.7% *Burkholderia pseudomallei* (95%CI: 3.7-5.9), 3.3% *Moraxella catharrhalis* (95%CI: 1.3-8.3), 2.8% *Kluyvera spp.* and *2.8% Serratia plymuthica /marcescens* (95%CI: 1.1-7.0), 2.5% Mycoplasma pneumonia (95%CI: 0.9-7.1), 1.6% *Treponema pallidum* (95%CI: 1.1-2.5*)* and 0.7% *Enterobacter intermedium,* 0.7 *Enterobacter aerogenes* and 0.7% *Escherichia hermannii* (95%CI: 0.1-3.9)), 1.1% *Yersinia pestis*(95%CI 0.7-1.8).
Helminths detected included: 19.3% *Ascaris lumbricoides* (95% CI: 14.2-25.8); 10.8% hookworms (95% CI: 7.0-16.3); 6.3% *Schistosoma mansoni* (95% CI: 3.5-10.8); 1.1% had *Schistosoma haematobium*; 1.2% *Strongyloides stercoralis* (95% CI: 0.3-4.1); and 2.8% had *Trichuris trichiura* (95% CI: 1.2-6.5). It is worthy to note that these helminthes are collectively neglected tropical diseases and also known as diseases of poverty.

Though malaria remains endemic, the results provide evidence of several other pathogens in circulation in Bo, Sierra Leone, one of which, Chikungunya, has a higher prevalence (46%) than malaria (23%). Among the bacteria, *Salmonella enterica* serotype Typhi is of importance as the population antibody levels has risen such that three-fifth of the study population had up to 1:120 titers of both Anti-O and Anti-H antibodies. A new cut-off point for the Widal test at about 1:160 or above is recommended to prevent over prescription of antibiotics for cases not related to typhoid. This study demonstrates the need to prioritize diagnosis and treatment of NMFIs in Sierra Leone.

DEDICATION

This work is dedicated to my late mother Madam Angella Miatta Koroma (RIP), whose love and care raised me up to be the man that I am today. My maiden introduction to resource poverty, sanitation and health systems were imbibed from my mother who worked as a rural development worker in very resource-poor environments in Sierra Leone. My mother helped organize local communities and donors to build schools, bridges, clinics and latrines. During those times, I accompanied her if her visits were on weekends, since she needed help with her motor bike, which she had used for almost a decade. We slept on beds that were infested by bed bugs in villages where help was direly needed.

My mother was not fortunate to have a university degree, and she always commented that I shall acquire the education that she never could acquire. It is for this purpose that I hereby dedicate this thesis to my late mother, a real mother that can be emulated across the globe.

DECLARATION

I **Rashid Ansumana**, declare that this thesis has been composed by me under the supervision of Moses J. Bockarie and Russel Stothard Jr.

Additional supervisory support was provided by Mary H. Hodges and Aiah Gbakima in Sierra Leone

Information from other sources have been cited or indicated in the thesis. This thesis has not been submitted in another form for any other degree or professional qualification.

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LIST OF ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
AnGap	Anionic gap
API	Analytical profile index
ARI	Acute respiratory infections
BCID	Blood culture identification panel
BT	Biothreat panel
BUN	Urea nitrogen
CCHF	Crimean-Congo Hemorrhagic Fever
cDNA	Copy DNA
ChikV	Chikungunya virus
CHEM8+	iStat Catridge for 8 blood chemistry parameters
DNA	Deoxyribonucleic acid
FA	FilmArray
FBC	Full blood count
FRP	FilmArray Respiratory Panel
FDA	Food and Drugs Administration
GDP	Gross Development Product
HAV	Hepatitis A Virus
HBcAB	Hepatitis B core antibody
HBeAB	Hepatitis B envelope antibody
HBeAg	Hepatitis B envelope antigen
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCT	Hematocrit
HCV	Hepatitis C Virus

HDI	Human Development Index
HIV	Human Immunodeficiency Virus
ICT	Immunochromatographic test
IGM	Immunoglobulin-M
IGG	Immunoglobulin-G
IL-1	Interleukin-1
IL-6	Interleukin-6
K	Potassium
LFI	Lateral Flow Immunoassay
LPS	Lipopolysaccharide
LRT	Lower respiratory tract
MOHS	Ministry of Health and Sanitation
Na	Sodium
NTD	Neglected tropical disease
NMFI	Non malaria febrile illness
PCR	Polymerase chain reaction
PCV	Hematocrit
PPP int.	International purchasing power parity
RLFI	Rapid Lateral Flow Immunoassay
RNA	Ribonucleic acid
RPM	Resequencing pathogen microarray
RSV	Respiratory Synctial Virus
SSA	Sub-Saharan Africa
SS	Salmonella shigella agar
TB	Tuberculosis
TCO2	Total carbon dioxide
TEI	Tropical emerging infections
Tet-X	A gene that encodes for resistance to tetracycline
T1	Tier 1
T2	Tier 2

USD	US Dollars
VCT	Voluntary counselling and testing
WBC	White blood count
TSA	Tryptic soy agar
URT	Upper respiratory tract
20E	20 enterobacteriaceae

CHAPTER ONE

INTRODUCTION

Fever and febrile illnesses account for a great burden of diseases in resource-poor countries. This chapter introduces a thesis on the tiered laboratory analyzes for common infections to characterize febrile morbidity not related to malaria in Sierra Leone. The section defines fever and febrile illnesses and briefly overviews the burden of malaria and non-malarial febrile illnesses in sub-Saharan Africa (SSA) in concert with diagnostic challenges within SSA including over-diagnosis and misdiagnosis. The study country Sierra Leone is described with administrative features, socio-demographic characteristics, health service utilization and access to healthcare highlighted. The study objectives, hypothesis and research questions and a generic method for the study are included. The final section describes the thesis structure.

1.1.0 Characteristics of Fever and Febrile Illnesses in Africa

Fever or pyrexia is usually a consequence of infection [1,2] and is a prominent clinical indicator of infectious disease processes in humans.[3] It is also an adaptive compensatory defense mechanism that activates the immune system; resulting in a decrease in pathogen growth rate and an increase in host survival.[4] Fever which is a regulated increase in body temperature above normal is distinct from hyperthermia[5] that may result from physical exercises. The increased metabolic heat production during exercise causes hyperthermia and is reversed by the negative-feedback loop for thermoregulation unlike fever[6], marked by a change in thermal preference and relative independence from ambient temperature.[7]

Due to variations in body temperature, no single value can be defined as fever[8]. However, febrile temperatures start from 37.5 degrees Celsius or greater without the use of fever-reducing medications[9] and include: rectal temperature above 37.5ºC; single oral temperature above 37.8ºC or repeated temperature of 37.2 ºC[10]; armpit temperature above 37.2ºC(for

newborns), ear temperature above 38ºC[11]. Accepted values for fever from previous studies are summarized in the table below:

Table 1.1: Overview of Fever Temperatures

Definition of Fever	**Reference**
>37.5 ˚C (oral)	Watt & Jongsakul, 2003
37.8 ˚C (oral)	Polat *et al*, 2014[12]; Yoke *et al* 2009[13]
≥38 ˚C (oral)	Manock et al. 2009; Murdoch et al., 2004
>38 ˚C (axillary)	Blacksell *et al.* 2007; Nga *et al.*, 2006, Wagenaar *et al.*, 2004
≥38 ˚C (unspecified)	Ellis *et al.* 2006; Kasper *et al.*, 2010
> 38.3 ˚C (oral)	Leelarasamee et al. 2004;
> 38.3 ˚C(unspecified)	Chrispal *et al.* 2010,
unspecified	Joshi *et al.* 2008; Suttinont *et al.* 2006

Adapted from Baily[14]

Moreover, fever occurs at a cost to the body such that for every degree increase in body temperature about 11-13% of oxygen is demanded, commensurate with the increase in metabolic processes[15]. Further increase in body temperature may have deleterious effects on the neuronal system and sometimes results in fatality [5]. Typically a variety of exogenous and endogenous substances with pyrogenic and antipyretic properties initiate, characterize, regulate and terminate febrile episodes.[4,7] Pyrogens induce fever and are exogenous when formed outside the host. Microbes, their secretions and materials such as lipopolysaccharides (LPS) are exogenous pyrogens.[3] Endogenous pyrogens, formed inside the host, include cytokines such as interferon gamma, ciliary neurotrophic factor, tumor necrosis factor and interleukins (IL) (Eg IL-6 and IL-1).[4,3,16] IL-1 also promotes leukocytosis, activate lymphocytes, and stimulates prostaglandin synthesis; and in concert with other cytokines, reduce serum iron and zinc, heighten the production of acute phase proteins in hepatocytes, and induces muscle proteolysis among other functions in many different tissue systems to produce a range of metabolic and hematologic effects including fever, called “acute phase response”[16].

Due to increasing temperature during febrile illnesses, certain disease-causing organisms

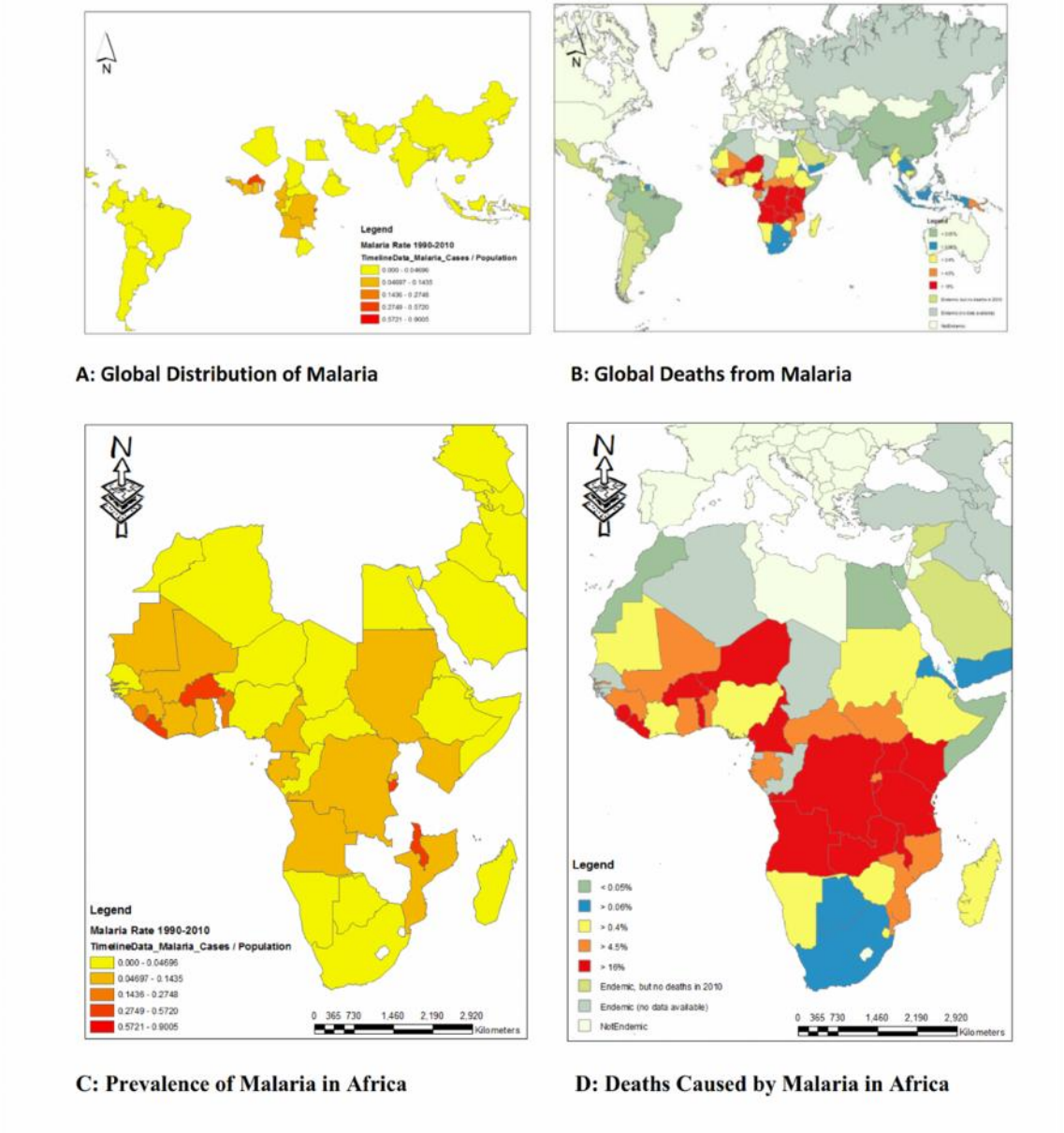
lose their disease-causing capability while enabling host defense mechanisms.[17] At febrile temperatures, IL-1 reduces iron and zinc in serum; iron is needed by bacteria to replicate using iron-binding "siderophores" that are also temperature-sensitive and suppressed at elevated temperatures[5],[18]. In the same vein, viral replication is inhibited by destabilizing viral polymerase promoter complexes that in turn inhibits the syntheses of certain proteins required for the synthesis of viral particles in Influenza A and Polio[5].

1.1.1 Burden of Malaria in Africa

In tropical Africa, fever is commonly associated with malaria that was known variously as Roman fever, marsh fever[19] and whose name was derived from the Italian 'Mal=bad, Aria=air.'[20] Malaria is caused by Five species of the plasmodium parasite: *P. falciparum, P. vivax, P.ovale, P. malariae and P. knowlesi* all of which are transmitted by the female anopheles mosquito, which is the vector of the parasite.

Over 2.4 billion people are at risk of *P. falciparum* infection, which result in about 300 to 500 million clinical episodes and 1million deaths annually[21]. While about 2.9 billion persons are at risk for *P. vivax* infection with up to 300 million clinical episodes per year[21]. A vast proportion of malaria morbidity occurs in sub-Saharan Africa, SSA(Fig 1.1).

FIG 1.1: MAP SHOWING SSA WITH HEAVIEST MALARIA BURDEN



1.1.2 Non-malaria Febrile Morbidity

The high morbidity of malaria in sub-Saharan Africa tends to eclipse other febrile illnesses that are also common and co-exist with malaria. These fevers caused by endogenous and exogenous pyrogens not related to malaria (non-malaria febrile morbidity) are common in tropical and subtropical countries. They may be attributable to multiple etiologic agents including viral, bacterial and parasitic infections in countries, which are resource-poor and endemic for malaria[22].

Typically several pathogens contribute to the non-malaria febrile morbidity in SSA. In a study of the etiology of acute febrile episodes in children in Tanzania, Mahende *et al.*[23] reported 46.8 %(406/867) prevalence of upper respiratory tract infections, mainly of viral etiology[24]; 15% pneumonia; 21.2% gastroenteritis, 8.3% malaria, 17.7% bacterial infections and 1.2% HIV infection. In a related study, Crump *et al.*[25] reported 1.6%(14/870) of malaria as the cause of actual fever while by contrast, bacterial, mycobacterial, and fungal bloodstream infections accounted for 9.8%, 1.6%, and 2.9% febrile admissions, respectively. As an addendum, Animut *et al*[26] in Ethiopia, reported 7% pneumonia, 5.8% typhoid, 5.1% typhus and 2.6% brucellosis as contributors to non-malarial febrile morbidity.

Several other studies in Africa reported a range of viral etiologic agents for non-malaria febrile morbidity. Ouedraogo *et al*[24] in Burkina Faso, reported 73%(153/203) of acute respiratory children with at least one virus and detected 175 viruses: human rhinoviruses(42.1%), enteroviruses(18.2%), respiratory syncytial virus(RSV)(12%), influenza virus(6.2%) and also bacteria(30%). Other viral illnesses in the disease ecology include: Crimean-Congo hemorrhagic fever(CCHF)[27], O'nyong-nyong virus[28] chikungunya virus across Africa[29], Lassa fever in West Africa[30], dengue fever[31] , viral hepatitiss[32], HIV[33], Ebola[34,35] and Maburg[36] among others.

Further, neglected infections cause a high morbidity in SSA affecting over 200 million persons within SSA. Prevalence reported include: 29% hookworms[37], 25% schistosomiasis[37], 25% ascariasis[38], 24% trichuriasis[38], 6-9% lymphatic filariasis[37], 5% onchocerciasis[39] and

3% active trachoma[37] among others. The NTDs cause a great deal of disability exacerbating poverty in these regions.

1.1.3 Diagnostic Challenges by Non-Malaria Febrile Illnesses in Resource-Poor Malaria Endemic Regions.

NMFIs pose challenges[40] to peripheral health systems in resource-poor countries, such that they are clinically under-diagnosed while malaria remains over-diagnosed[41,42,43]. Misdiagnoses of a febrile condition may lead to the wrong prescription that delays treatment and increases expenditure on health-care and also leads to increased morbidity and mortality. Misdiagnoses of malaria could mean failing clinically to suspect malaria as a cause of febrile illness; leading to deaths in non-malaria endemic countries. [44, 45,46] In SSA misdiagnoses occur from over suspicion of malaria for non-malarial febrile cases due to the low specificity of predictors of malaria [47,48] which also overlap with several other febrile illnesses. [49, 50, 42]

A typical example of misdiagnoses, consequent treatments, and their effects was illustrated by the yellow fever outbreak in Uganda in 2010. The outbreak attributed to Ebola virus in early October 2010 was misdiagnosed as amoebic dysentery, alcohol poisoning, and *Yersinia pestis* before being accurately diagnosed as yellow fever after three months.[51]Case fatality rate was 25% (50/200) by the time of confirmation.[51]

1.1.4 Home-based and Presumptive Diagnoses of Febrile Illness

Additionally, for most SSA countries including Sierra Leone, over 70% of febrile cases are diagnosed at home in proxy of or prior to clinical or laboratory diagnoses.[49] There is an increased tendency for fatality and many people die each year without any clinical investigation, but the deaths are attributable to infectious diseases.[52] For example, Roth *et al*[53] working on the factors associated with mortality in febrile patients in Kenema, Sierra Leone reported 53.4%(229/429) of mortality were of unknown causes. Such a trend has been the case for decades in Africa,[54,49] until the recent WHO policy of test-treat-and-track. The policy was a consequence of substantial evidence that the intensity of malaria transmission in Africa is declining[55,56,57,58,59]and rapid malaria parasitemia tests are well distributed in endemic countries and easy to use.[56] In addition, presumptive treatments are associated with an

increased cost of drugs with potentially more risk from the medicines and a tendency for drug resistance.[49,57]

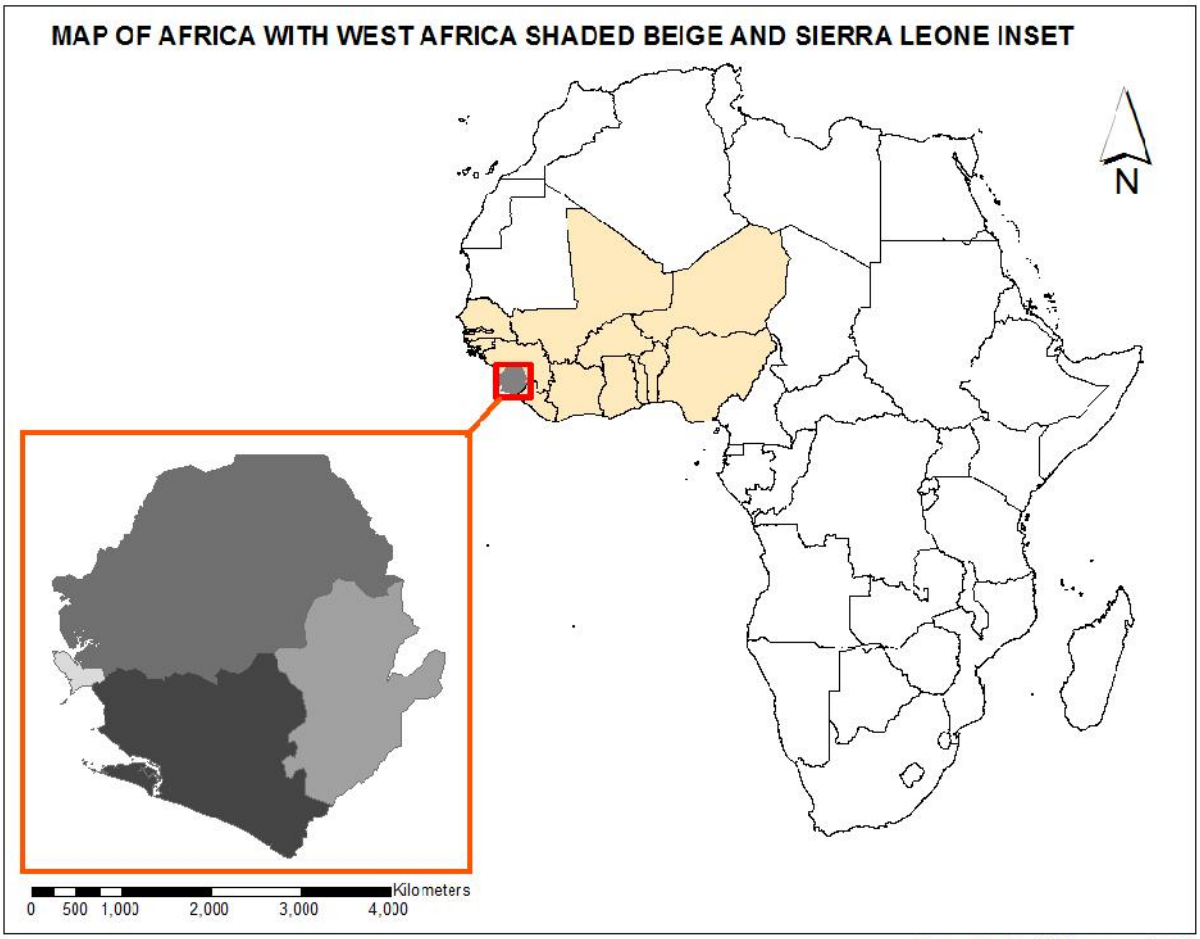
1.1.5 Malaria Over-diagnosis

Notwithstanding, several instances of clinical or laboratory over-diagnoses of malaria have been reported in the literature. In a study in Tanzania, East Africa, involving 4,474 febrile patients; 54% were film negative for malaria, only one-third of these were treated with antibiotics and the fatality rate of non-malarial cases was 12%[42].

In North Africa, A-Elgayoum, *et al.*[43] in a retrospective study of 3203 blood smears from 95 peripheral health facilities in Sudan, reported, 75.6% of over-diagnosis. A preceding study by Hume *et al.*[49] assessed the household cost of malaria overdiagnosis in rural Mozambique, South Africa and reported clinical over-diagnoses of 23% in children under 16years and 31% in adults with malaria. Similar results have been reported in other studies in Africa. In Malawi,[49] 23% fatality of clinically diagnosed cerebral malaria occurred from NMFI; in Kenya, 39% of co-morbid malaria patients died of non-malarial causes[60] and in another study, cerebrospinal fluid of 9% of 49 children with cerebral malaria revealed Herpes Simplex Type 1 infection[61].

In Sierra Leone, West Africa(Fig 1.2), a study conducted in Bo, in 2005, reported 18% over-diagnoses of malaria.[62] Another study in the same area on pediatrics between 2004 to 2006 reported 35% of over-diagnoses of malaria[63]. Similarly, Evans *et al*[64] in Ghana, reported 40% bacteremic cases, clinically diagnosed as severe malaria, with a case fatality rate of 39%.

Fig. 1.2 Map of Africa Showing West Africa and Sierra Leone Inset



1.1.6 Problems Associated with Testing and Treating Febrile Illnesses in SSA

Moreover, the proliferation of rapid diagnostic tests for malaria in SSA have made it accessible to test-and-treat and to exclude NMFI from clinically diagnosed malaria cases[65]. However, several factors make it difficult also to test and treat NMFIs. Clinical laboratory services are among the most neglected areas in SSA, having inadequately trained staff, fewer

resources, fewer standardized infrastructure and inadequate equipment[66]. Reagents, if available, may not be adequately stored due to epileptic power supplies[67]. Moreover, restocking supplies locally is almost impossible for many ice-dependent consumables. The cost also remains a significant issue to test for NMFIs, caused by several pathogens, requiring sometimes multiplex molecular diagnostics. Such diagnostics are mostly not affordable even at a cost recovery price, as patients in these countries mostly leave under 2USD a day in many SSA countries. Global health care donations to SSA could solve this problem, but more attention is paid to disease prevention and treatment than to diagnostics[52]. Differential diagnoses of febrile illnesses remain eminent, but resource-poverty prevents the consistent application of this method. Moreover, in many poor countries of the world, febrile illnesses cause more problems in remote areas with less equipped laboratories and fewer experienced technicians[65]. The quality control, sensitivity and specificity of tests under these conditions are questionable and their use in the treatment still prone to high fatality rates[52].

Simpler, cost-effective tests with precision for different etiologic agents of febrile illnesses; requiring fewer skills for use and usable even in remote settings can salvage the situation. However, there are also several cheap tests with either very low sensitivity or low specificity making it had to depend on their results alone during the first time of use. Higher throughput assays that are expensive for distributed use may be essential for confirming discordant results of lower class tests or for guiding in assay selection.

1.2.0 SIERRA LEONE COUNTRY PROFILE

This Phd study was carried out in the Republic of Sierra Leone, which is on the West Coast of Africa bordering on Guinea on its north and Liberia on the south and the Atlantic Ocean on the West (Fig 1.2).

1.2.1 Administrative, Demographic, Socioeconomic and Health Profile of Sierra Leone

Sierra Leone is divided into four main administrative regions: Western Area, Eastern Province, Northern Province, and Southern Province. Western Area is subdivided into two administrative districts (Western Urban and Western Rural), the South has four subdivisions

(Bo, Bonthe, Moyamba and Pujehun districts) the East has three (Kenema, Kono and Kailahun) the North has five(Bombali, Tonkolili, Kambia, Port Loko and Koinadugu Districts). Each district is further divided into chiefdoms comprised of towns and villages. Each administrative unit has a unit head that oversees the administration of that unit.

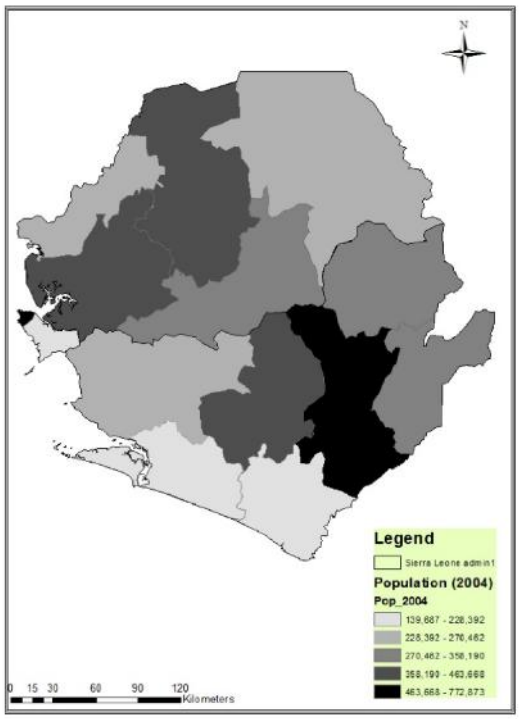
The overall population of the country was 5,997, 000 million inhabitants in 2011, with a median age of 18 and growth rate of 3.3 % [68]. Estimating from the 2011 figures the population in 2012 was 6,194,901. About 43% of the population are under 15 years of age, 828,114 under-five years of age, 1,661,845 are between 5 and 14 years of age and 4% over 60 years. A map of the Sierra Leone population is presented in Fig 1.3A. The population density was 86.7 per square kilometers. Further, urban centers account for 39.2% of the population, and the rural areas account for 60.8%, (Fig.1.3B). The crude birth rate of about 37.8/1000 population. The total fertility rate is 4.7 children per woman. The overall death rate is 20.6/1000 population, but in infants, the mortality rate is estimated at 103.5/ 1000 live births whereas the under-fives mortality rate is 202/1000[69]. Maternal mortality is very high at 1600/100,000 live births. Malnutrition in the country ranges from 4 to 9 % (Fig 1.3C). Based on the Human Development Report [70]Sierra Leone is the 9th least developed country, with a human development index (HDI) of 0.359 ; almost one-third the value of the first country in the world, Norway, which has a HDI of 0.955. The GDP in 2011 was 2.973 billion US Dollars with a 6% annual growth rate [71].

Due partly to resource poverty(Fig 1.3D) exacerbated by the 11 years civil war that took place in the country from 1991 to 2002, details of the socio-economic profile of Sierra Leone are gloomy. The country had a crude birth rate of 37.8 per 1000; neonatal mortality rate of 49 per 1000, infant mortality rate of 119 per 1000 and under-five death rate of 185 per 1000 in 2011; making Sierra Leone one of the worst countries in the world to be a child under-five years of age [68].

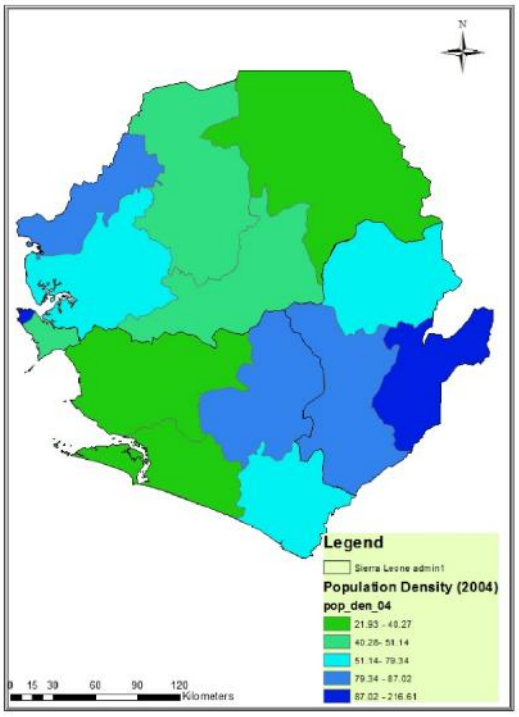
The literacy rate is 42% of adults aged 15 years and above. The gross national income per capita is 840 international purchasing power parity, USD (PPP.int. $) and 36 per 100 cellular subscribers. The out of pocket expenditure as a percentage of private expenditure on health was 91.4% in 2010 and 0.2% private prepaid plans as a percentage of private expenditure on health. The per capita total expenditure on health in 2010 was 171(PPP int. $) and the per

capita Government spending on health in 2010 was 26(PPP in $). About 20.8% of the gross domestic product, (GDP) was expended on health in 2010. The Government spends 15.3% of the total expenditure on health in the country, and private expenditure on health was 84.7%.

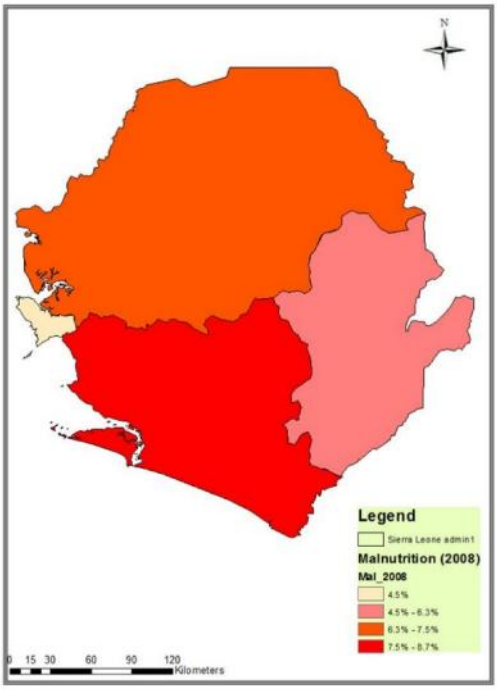
Fig 1.3: Population, Poverty and Malnutrition Maps of Sierra Leone



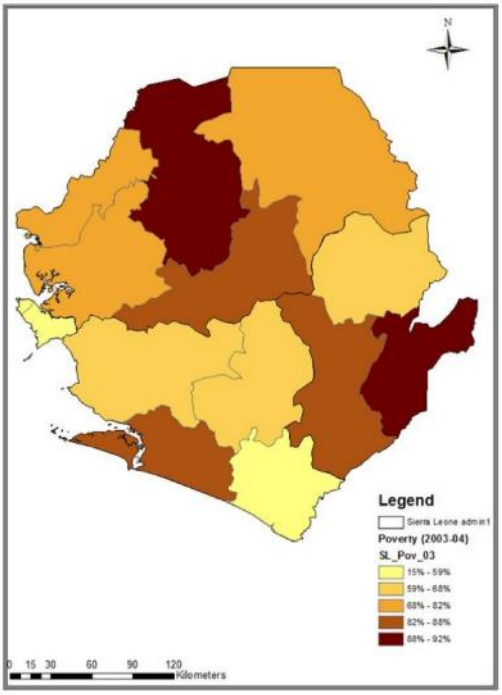
A: POPULATION DISTRIBUTION OF SIERRA LEONE



B: POPULATION DENSITY OF SIERRA LEONE



C: MALNUTRITION IN SIERRA LEONE



D: DISTRIBUTION OF POVERTY IN SIERRA LEONE

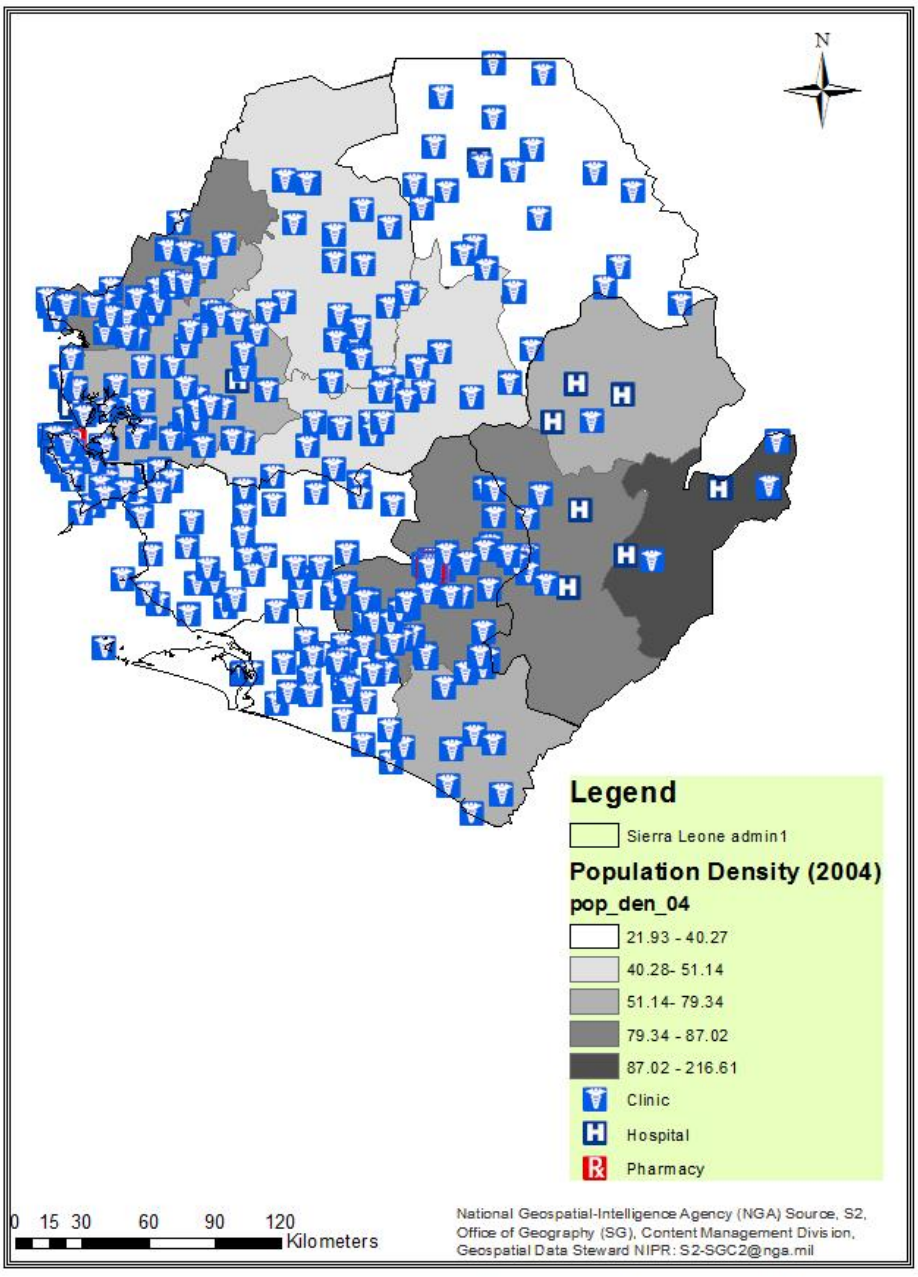
Health Service Utilization in Sierra Leone is useful for antenatal care. About 75% get prenatal care in over four visits per year. At least 61% have births attended by skilled health personnel, and 80% of 1-year olds get measles immunization, and there is 87% smear-positive TB treatment success[72], which was 79% in the year 2000, but TB detection rate is 52%[73].

However, there were 0.2 physicians and 1.7 nurses per 10,000 population in 2010 when SSA regional averages were 2.5 and 9.1 respectively. The Death of children under five is attributable to malaria(23%), pneumonia(17%), diarrhea(12%) and other diseases (16%)[68]. For the general populace, the causation of death in the country is mainly due to communicable diseases(85%) while non-communicable diseases account for 10%.[74] There is a high risk of high blood pressure, 41.9% for males and 40.5% for females and also a high likelihood of tobacco use. Under 20% of the population has access to sanitation and a little over 50% have access to clean water [74].

Access to healthcare is hindered further by long distances people have to cover to visit a healthcare center within the country. A map of the locations of healthcare centers with a background of the population density is presented below (Fig. 1.4).

Malaria remains endemic in Sierra Leone. In 2011, Sierra Leone reported more cases of malaria, 638 859 cases, to the WHO than any other country in the World. There were 810 cases of HIV/AIDS per 100,000 population and 1,372 tuberculosis per 100,000 population[68]. There are also possibilities of multiple comorbidities with malaria, and little has been reported about co-morbidities of malaria with non-malarial febrile illnesses. In peripheral health centers in Sierra Leone, it is hard to determine, other causes of infections if malaria is not a cause of an infection.

Fig 1.4: Health Service Distribution Overlaid on Population Density



1.3 OVERVIEW OF FEBRILE ILLNESSES IN SIERRA LEONE

Several febrile illnesses have been reported in Sierra Leone, but predominant among them is malaria. Winkler *et al*[75] reported 74%(423/591) malaria prevalence in children in Northern Sierra Leone and a related study[76] in the same area also reported 73% prevalence of malaria. However, Nnedu *et al*[62] reported an 18% over-diagnoses of malaria in southern Sierra Leone and another study in the same area on pediatrics between 2004 to 2006 reported 35% of over-diagnoses of malaria[63].

Roth and colleagues[53] recently reported 53% undifferentiated febrile illnesses in Kenema, Eastern Sierra Leone. Schoep *et al*[77] reported between 60-70% lassa-like, undifferentiated febrile illnesses.

Reported prevalence of NMFIs include 5.1% pneumonia[53], 4.9% Lassa fever[53], 41.7% hookworms[76], 8.3% *S. mansoni* infections[76], 1.7% *A. lumbricoides*[76] and 2.2% *T. trichiura*[76], 4.3% Dengue[77], 4% Chikungunya[77], 2.8% West Nile Virus[77], 2.5 % yellow fever[77], 2% Rift valley fever virus[77], *8.6% Ebola*[77] *and 3.6% Maburg*[77]. Other diseases implicated in the febrile ecology in Sierra Leone include Typhoid fever, [78] acute respiratory infections,[79] Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis E [80,33,81,] HIV[82], tuberculosis[83], lymphatic filariasis[84,85] onchocerciasis[86], schistosomiasis[87], Lassa fever[88] and yellow fever[89].

1.4 THE PRESENT STUDY

1.4.1 Aim of the Study

The primary objective of this Ph.D. study was to investigate the prevalence/incidence of non-malarial febrile infections in Bo, Sierra Leone, using a tiered laboratory analyzes method.

1.4.2 Specific Objectives

The specific objectives were to:

Investigate the types and etiology of non-malarial febrile illnesses in Bo, Sierra Leone.
Determine the prevalence/incidence of non-malarial pathogens causing febrile illnesses.
Investigate the distribution of non-malarial febrile illnesses using, a cross-sectional qualitative survey combined with a spatiotemporal febrile syndromic surveillance system involving Ushahidi.

1.4.3 Hypothesis

Malaria accounts for less than 50% of the febrile morbidity presented to peripheral health facilities in Sierra Leone.

1.4.4 Primary Research Questions

What Pathogens are common causes of non-malarial febrile illnesses in Bo?
What is the prevalence/incidence of non-malarial, febrile illnesses in Bo?
Can syndromic surveillance of non-malarial febrile illnesses in Bo be correlated with the clinical diagnosis?

1.4.5 Plan of the Study

The study was designed for one year, July 2012 to June 2013. A baseline syndromic survey prefaced the laboratory analyzes and determined the recruitment of subjects. Subjects recruited were followed for one year. A subsequent data collection and analyzes was also done in 2014. Subjects were recruited from the catchment population of Mercy Hospital described previously.[90], in brief, Mercy Hospital is a private hospital on the northern edge of Bo, Sierra Leone (Fig 1.5 and Fig 1.6).

Fig 1.5: Map of Sierra Leone with Bo District and Bo Town Insets

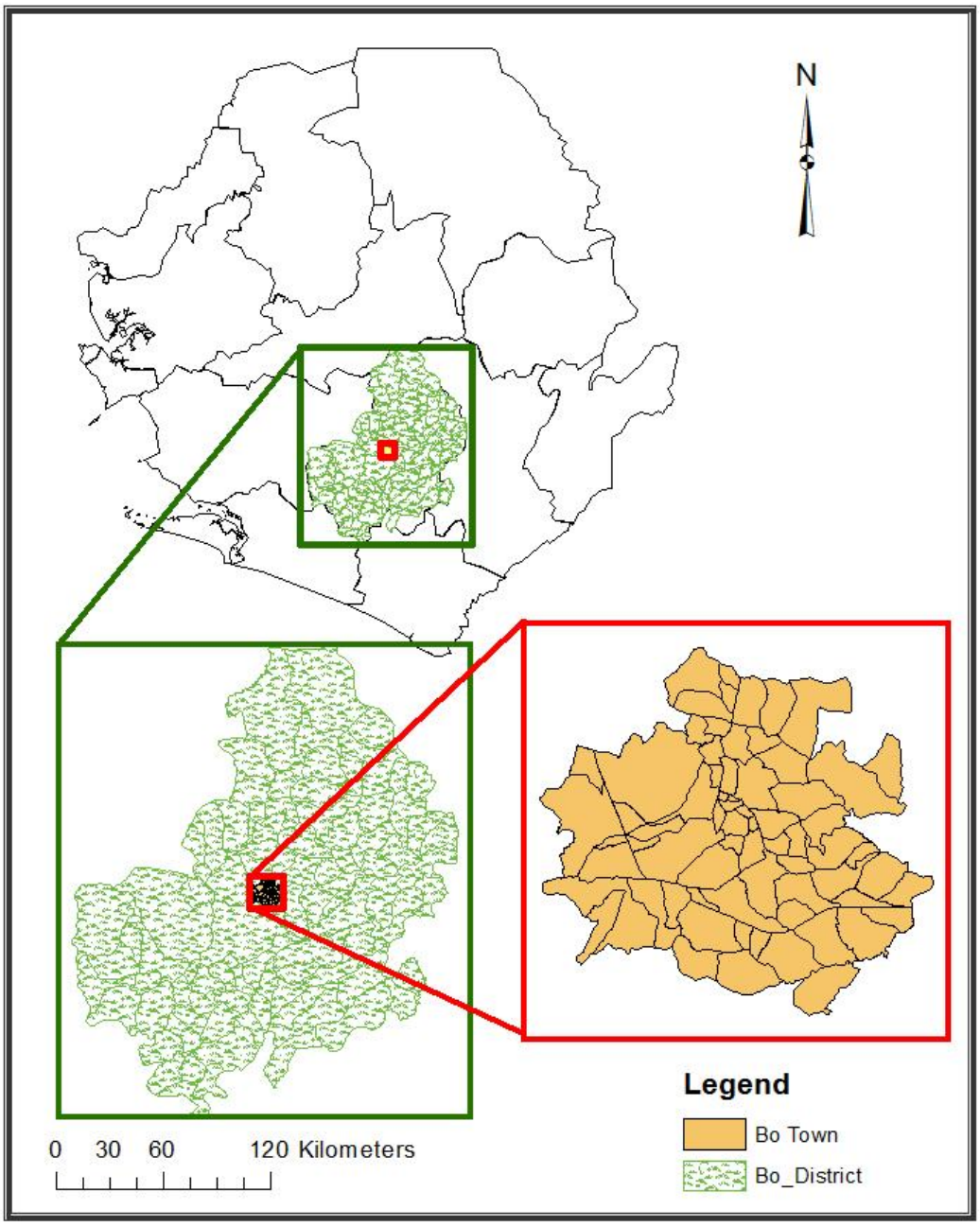
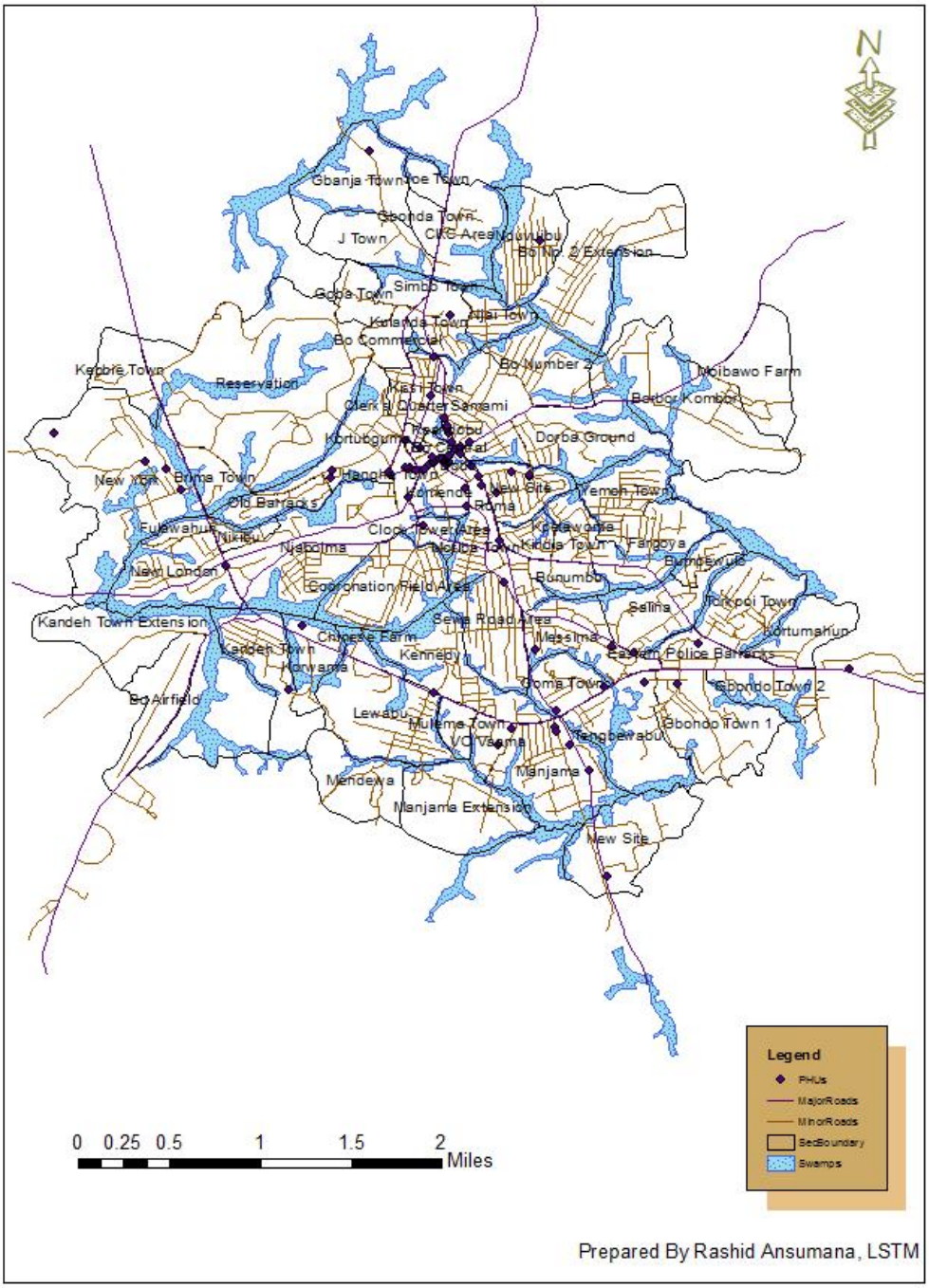


Fig 1.6: Map of Bo Town



In this Ph.D. research, a tiered laboratory analyzes of bio samples for pathogens at different cadres and costs of analyzes were used, with simpler, cheaper and readily accessible assays

at the lowest tier and broad-spectrum, decision-quality, throughput assays at the final tier, to facilitate: (a) a detection of uncommon pathogens, (b) informing about Tier1 and Tier Two assay selections and (c) discovering new or emerging pathogens.

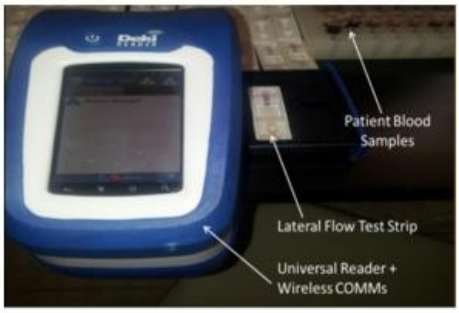
Biosamples of the subjects recruited, who reported with fever, were analyzed using lateral flow immunoassays (malaria, widal, Salmonella typhi IgG/IgM, Chikungunya, IgM), Dengue (NS1, IgG/IgM), Leptospirosis, HBV(HBsAg),HCV,HAV, Syphilis, HIV 1 /2, *Yersinia pestis*, *Bhukhoderia pseudomallei*, Lassa fever, Ebola Virus Disease. Other tests carried out were culture and molecular tests at a tier (Tier 2 and 3). The tests done by the rapid tests were uploaded to a cloud database. Also, syndromes were recorded on the Ushahidi website created for this study at www.mhrlsl.com/ushahidi.

1.5 Overview of Research Methods

A baseline and syndromic survey were carried out of all households in the study community.

Fig 1.7: Survey Instrumentation

A: Survey tools



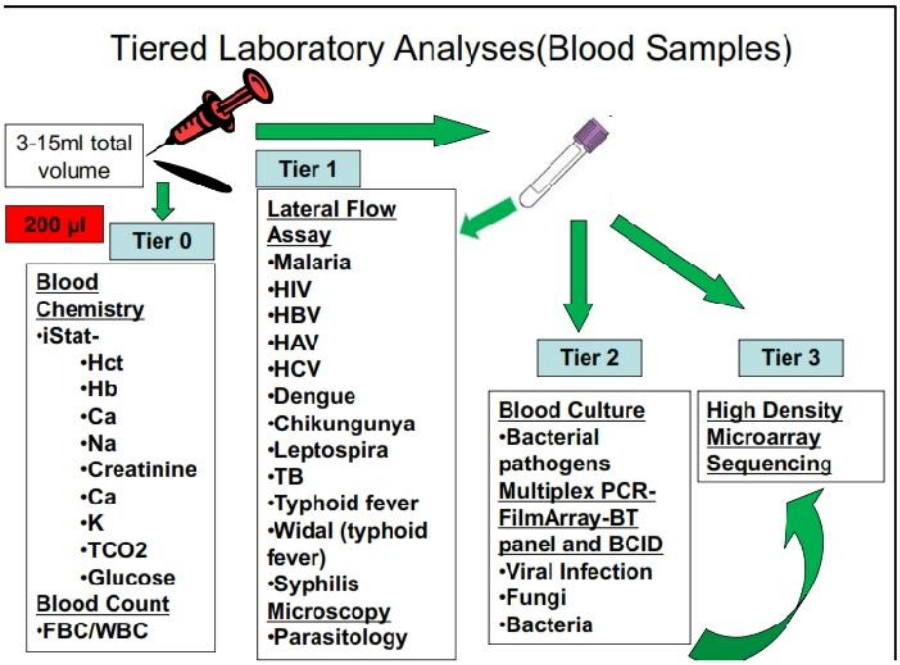
B: Deki Reader, Uploads LFI Results to Cloud Database

C: Filemaker Server for Synchronizing Patient Metadata

D: Results Uploaded on the Deki Reader

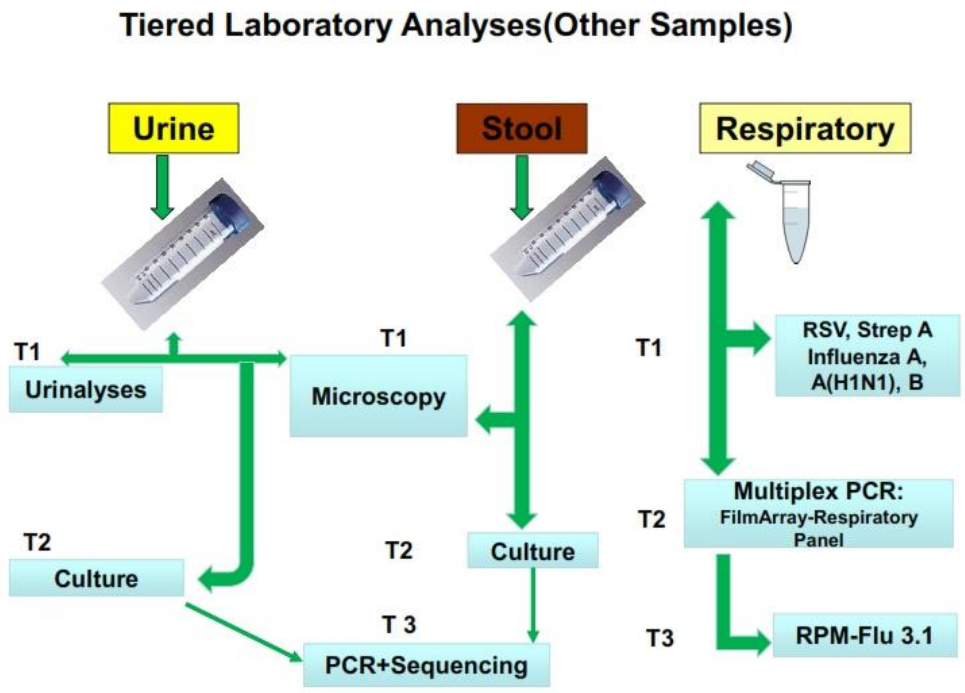
A cohort of 1403 persons were recruited from the catchment population of Mercy Hospital and followed for a period of one year. Biosamples were obtained from consented, febrile subjects and used for laboratory analyses involving three tiers. The first tier included use of rapid, lateral flow assays RLFAs) for chikungunya, malaria, typhoid fever, syphilis, HIV, hepatitis A,B and C, dengue fever, leptospirosis, influenza A and B, RSV, Strep A and TB(using LFIs from SD Bioline). Widal agglutination tests were done with the febrile antigen kit (to assess the antibody levels of *Salmonella enterica typhi*.

Fig 1.8: TLA Flow Chart for Blood Samples



Urinalyses and blood chemistry analyses were done using the iStat 300 analyzer for blood chemistry analyses and the urine dipstick, Multistix® 10SG (Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA) for urinalyses. Urinalyses also entailed centrifugation and microscopic smear with the Bayer Atlas of Urine Sediments (Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA). Stool samples were assessed microscopically for parasites including helminths.

Fig 1.9: TLA Flow Chart for Urine, Stool and Nasal or Throat Swabs



Subsequent tests included microbial culture using blood, urine or stool samples. Blood culture was done using the Oxoid SIGNAL (Oxoid Ltd., Basingstoke, United Kingdom) blood culture system. Urine and stool samples were grown on agar media (Hardy Diagnostics, Santa Maria, CA) or CHROMagar™ plates (CHROMagar, Paris, Fr.) which are powder based media with agar, peptone and yeast extracts and chromogenic mix for differentiation or group identification. The other types of media used were: Brain Heart Infusion (BHI) Agar, Tryptic Soy Agar (TSA), MacConkey agar, Hektoen enteric (HE) agar, Salmonella Shigella Agar (SS) (Hardy Diagnostics, Santa Maria, CA). API20E, CHROMagar plates and PCR were used for identifying colonies from culture media.

Advanced molecular tests done included: the resequencing pathogen microarray system RPM-TEI v. 1.0 and RPM Flu v3.1 described previously by Leski et al[91,51]. Additionally, the multiplexed PCR in vitro diagnostic system, FilmArray (BioFire Defense, Salt Lake City, UT, USA) that is also a lab-in-a-pouch system that performs a mesoscale fluid manipulation (working volume of 0.001 to 0.2ml) in a disposal, self-contained, thin-film plastic[92] pouch[93], was used with panels(http://filmarray.com/the-panels/) that detect respiratory pathogens, blood culture identification panels detecting pathogens associated with sepsis and biothreat pathogens (http://www.biofiredx.com/pdfs/FilmArray/Infosheet,%20FilmArray%20Biothreat-0039.pdf) such as *Ebola zaire* and *Yersinia pestis.* Culture isolates were identified genetically after PCR amplification and sequencing of a 16S rDNA amplicon that spanned the V3 and V4 variable regions and the rpoB or gyrA gene previously described[94,95,96]. Additionally, antimicrobial determinant microarray was used.

A local area network was created using a Eugenius Router and a FileMaker server 12 was established to synchronize patient metadata per visit. A crowd sourcing and crowd mapping site was created using Ushahidi and Bluehost at ww.mhrlsl.com/GIA/ushahidi for daily incidence of diseases which were geolocated. A cloud database was set up for Tier 1 tests using a Deki Readers (Fio Corporation, Canada).

1.6 STRUCTURE OF THESIS

The thesis is comprised of eight chapters.

Chapter 1 is the General Introduction and Aim of the Study. The chapter introduces fever, febrile illnesses and non-malaria febrile illnesses in resource-poor countries. It describes the diagnostic problems in resource-poor countries and also describes the study location and what this PhD study is about.

Chapter 2 is based on the survey that prefaced the laboratory data collection and analyses. The title is: Common Infections in Sierra Leone: Presumptive Self-diagnosis of malaria and other febrile illnesses in Sierra Leone.

Chapter 3 is titled-Common Infections in Sierra Leone: Viral Hepatitis.

Chapter 4 is titled-Common Infections in Sierra Leone: Respiratory Infections.

Chapter 5 is titled-Common Infections in Sierra Leone: Re-emergence of Chikungunya in Bo, Sierra Leone.

The Chapter is titled-Common Infections in Sierra Leone-HIV Infections.

Chapter 7 is titled-Common Infections in Sierra Leone-Undifferentiated Febrile Illnesses.

Chapter 8 is the General Summary, Conclusion, and Recommendations.

1.7 REFERENCES

1. Bartfai T, Conti B. Fever. ScientificWorldJournal 2010;10:490–503.

2. Mackowiak PA. Fever: blessing or curse? A unifying hypothesis. Ann Intern Med 1994;120:1037–40.

3. Ogoina D. Fever, fever patterns and diseases called “fever” - A review. J. Infect. Public Health. 2011;4:108–24.

4. Mackowiak PA. What we do when we suppress fever. Curr. Infect. Dis. Rep. 2005;7:1–4.

5. Cannon JG. Perspective on fever: The basic science and conventional medicine. Complement. Ther. Med. 2013;21.

6. Boulant JA. Role of the preoptic-anterior hypothalamus in thermoregulation and fever. Clin Infect Dis 2000;31 Suppl 5:S157–61.

7. Moltz H. Fever: Causes and consequences. Neurosci. Biobehav. Rev. 1993;17:237–69.

8. Mackowiak PA. Concepts of fever. Arch Intern Med 1998;158:1870–81.

9. Mackowiak PA, Bartlett JG, Borden EC, et al. Concepts of fever: recent advances and lingering dogma. Clin. Infect. Dis. 1997;25:119–38.

10. High KP, Bradley SF, Gravenstein S, et al. Clinical practice guideline for the evaluation of fever and infection in older adult residents of long-term care facilities: 2008 Update by the infectious diseases society of America. J Am Geriatr Soc 2009;57(3):375–94.

11. Jarrette RR. Pediatric House Calls:Treating Fever in Infants, Children and Teens [Internet]. 2013 [cited 2015 Apr 2];Available from: http://pediatric-house-calls.djmed.net/treating-fever-in-infants-children-and-teens/

12. Polat M, Kara SS, Tezer H, Tapısız A, Derinöz O, Dolgun A. Original Article A current analysis of caregivers ’ approache s to fever and antipyretic usage.

13. Yoke PK, Ma S, Seo YC, Chia FML, Kam KL. A cross-sectional study on reference ranges of normal oral temperatures among students in Singapore. Ann Acad Med Singapore 2009;38(7):613–20.

14. Bailey MS. Febrile Illnesses at the Colombo North Teaching Hospital in Sri Lanka. 2012;

15. Bruder N, Raynal M, Pellissier D, Courtinat C, François G. Influence of body temperature, with or without sedation, on energy expenditure in severe head-injured patients. Crit Care Med 1998;26:568–72.

16. Ogoina D, Onyemelukwe G, Musa B, Babadoko A. Seroprevalence and determinants of human herpes virus 8 infection in adult Nigerians with and without HIV-1 infection. Afr Health Sci 2011;11:158–62.

17. Mackowiak PA, Wasserman SS, Levine MM. An analysis of the quantitative relationship between oral temperature and severity of illness in experimental shigellosis. J Infect Dis 1992;166:1181–4.

18. Pieracci FM, Barie PS. Diagnosis and management of iron-related anemias in critical illness. Crit Care Med 2006;34:1898–905.

19. Rocco F. The miraculous fever-tree : malaria, medicine and the cure that changed the world. LONDON, UK: HarperCollins; 2003.

20. Prakash, Anupam;Saxena, Pikee;Nigam A. Disease remains unconquered, its name reminisces a “feel bad” atmosphere- “mal” “aria.” Indian J Med Spec 2013;4(1):5–9.

21. Bousema T, Drakeley C. Epidemiology and infectivity of Plasmodium falciparum and Plasmodium vivax gametocytes in relation to malaria control and elimination. Clin Microbiol Rev [Internet] 2011;24(2):377–410. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3122489&tool=pmcentrez&rendertype=abstract

22. Naing C, Kassim AIBM. Scaling-up attention to nonmalaria acute undifferentiated fever. Trans R Soc Trop Med Hyg 2012;106:331–2.

23. Mahende C, Ngasala B, Lusingu J, et al. Aetiology of acute febrile episodes in children attending Korogwe District Hospital in north-eastern Tanzania. PLoS One 2014;9(8).

24. Ouédraogo S, Traoré B, Nene Bi ZAB, et al. Viral Etiology of Respiratory Tract Infections in Children at the Pediatric Hospital in Ouagadougou (Burkina Faso). PLoS One [Internet] 2014;9(10):e110435. Available from: http://dx.plos.org/10.1371/journal.pone.0110435

25. Crump JA, Morrissey AB, Nicholson WL, et al. Etiology of Severe Non-malaria Febrile Illness in Northern Tanzania: A Prospective Cohort Study. PLoS Negl Trop Dis 2013;7(7).

26. Animut A, Mekonnen Y, Shimelis D, Ephraim E. Febrile illnesses of different etiology among outpatients in four health centers in northwestern Ethiopia. Jpn J Infect Dis 2009;62(2):107–10.

27. Grard G, Drexler JF, Fair J, et al. Re-Emergence of Crimean-Congo Hemorrhagic Fever Virus in Central Africa. PLoS Negl Trop Dis [Internet] 2011;5(10):e1350. Available from: http://dx.plos.org/10.1371/journal.pntd.0001350

28. Bessaud M, Peyrefitte CN, Pastorino BAM, et al. O’nyong-nyong Virus, Chad. 2006.

29. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: A re-emerging virus. In: The Lancet. 2012. p. 662–71.

30. Richmond JK, Baglole DJ. Lassa fever: epidemiology, clinical features, and social consequences. BMJ Br Med J 2003;327:1271–5.

31. Guzman MG, Halstead SB, Artsob H, et al. Dengue: a continuing global threat. Nat Rev Microbiol 2010;8:S7–16.

32. Gallagher KM, Novak RT. Hepatitis, viral. In: International Encyclopedia of Public Health. 2008. p. 374–82.

33. Wurie I, Smart J, Brown D. HIV and hepatitis B (HBV) coinfection - a gap in HIV treatment package in developing countries. XVII Int. AIDS Conf. 38 August 2008 Mex. City Mex. 2008;

34. Ansumana R, Bonwitt J, Stenger DA, Jacobsen KH. Ebola in Sierra Leone: A call for action. Lancet. 2014;384(9940):303.

35. Ansumana R, Jacobsen KH, Sahr F, et al. Ebola in Freetown area, Sierra Leone--a case study of 581 patients. N Engl J Med [Internet] 2015;372(6):587–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25539447

36. Bricaire F, Bossi P. Emerging viral diseases. Bull Acad Natl Med 2006;190(3):597–608; discussion 609, 625–7.

37. Hotez PJ, Kamath A. Neglected tropical diseases in sub-Saharan Africa: Review of their prevalence, distribution, and disease burden. PLoS Negl. Trop. Dis. 2009;3(8).

38. De Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: Updating the global picture. Trends Parasitol. 2003;19(12):547–51.

39. Molyneux DH, Hotez PJ, Fenwick A. “Rapid-impact interventions”: How a policy of integrated control for Africa’s neglected tropical diseases could benefit the poor. PLoS Med. 2005;2(11):1064–70.

40. Crump JA, Youssef FG, Luby SP, et al. Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. Emerg Infect Dis 2003;9:539–44.

41. Koram KA, Molyneux ME. When is “malaria” malaria? The different burdens of malaria infection, malaria disease, and malaria-like illnesses. Am J Trop Med Hyg 2007;77:1–5.

42. Reyburn H, Mbatia R, Drakeley C, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. BMJ 2004;329:1212.

43. A-Elgayoum SME, El-Feki AEKA, Mahgoub BA, El-Rayah EA, Giha HA. Malaria overdiagnosis and burden of malaria misdiagnosis in the suburbs of central Sudan: special emphasis on artemisinin-based combination therapy era. Diagn Microbiol Infect Dis 2009;64:20–6.

44. Grobusch MP, Kremsner PG. Uncomplicated malaria [Internet]. In: Malaria: Drugs, Disease and Post-Genomic Biology. 2005. p. 83–104.Available from: <Go to ISI>://000233456400004

45. Askling HH, Bruneel F, Buchard G, et al. Management of imported malaria in Europe. Malar. J. 2012;11:328.

46. Bouchaud O, Mühlberger N, Parola P, et al. Therapy of uncomplicated falciparum malaria in Europe: MALTHER – a prospective observational multicentre study. Malar. J. 2012;11:212.

47. Y A A, A G, G Y. Misdiagnosis and overtreatment of clinical malaria cases and their implications in western Kenya highlands [Internet]. Am. J. Trop. Med. Hyg. 2009;81:195. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=70337155

48. Amexo M, Tolhurst R, Barnish G, Bates I. Malaria misdiagnosis: Effects on the poor and vulnerable. Lancet. 2004;364:1896–8.

49. Hume JCC, Barnish G, Mangal T, Armázio L, Streat E, Bates I. Household cost of malaria overdiagnosis in rural Mozambique. Malar J 2008;7:33.

50. Berkley J, Mwarumba S, Bramham K, Lowe B, Marsh K. Bacteraemia complicating severe malaria in children. Trans R Soc Trop Med Hyg 1999;93:283–6.

51. Leski TA, Ansumana R, Malanoski AP, et al. Leapfrog diagnostics: Demonstration of a broad spectrum pathogen identification platform in a resource-limited setting. Heal. Res. Policy Syst. 2012;10:22.

52. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. Clin Infect Dis 2006;42:377–82.

53. Roth PJ, Grant DS, Ngegbai AS, Schieffelin J, McClelland RS, Jarrett OD. Factors Associated with Mortality in Febrile Patients in a Government Referral Hospital in the Kenema District of Sierra Leone. Am J Trop Med Hyg [Internet] 2014;92(1):172–7. Available from: http://www.ajtmh.org/cgi/doi/10.4269/ajtmh.14-0418

54. English M, Reyburn H, Goodman C, Snow RW. Abandoning presumptive antimalarial treatment for febrile children aged less than five years--a case of running before we can walk? PLoS Med [Internet] 2009 [cited 2014 May 1];6(1):e1000015. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2613424&tool=pmcentrez&rendertype=abstract

55. Snow RW, Amratia P, Kabaria CW, Noor AM, Marsh K. The Changing Limits and Incidence of Malaria in Africa. 1939-2009. Adv Parasitol 2012;78:169–262.

56. Graz B, Willcox M, Szeless T, Rougemont A. “Test and treat” or presumptive treatment for malaria in high transmission situations? A reflection on the latest WHO guidelines. Malar J 2011;10:136.

57. D’Acremont V, Lengeler C, Mshinda H, Mtasiwa D, Tanner M, Genton B. Time to move from presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children with fever. PLoS Med [Internet] 2009 [cited 2014 May 1];6(1):e252. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2613421&tool=pmcentrez&rendertype=abstract

58. Guerra CA, Gikandi PW, Tatem AJ, et al. The limits and intensity of Plasmodium falciparum transmission: Implications for malaria control and elimination worldwide. PLoS Med 2008;5:0300–11.

59. Wang S-J, Lengeler C, Smith TA, Vounatsou P, Akogbeto M, Tanner M. Rapid Urban Malaria Appraisal (RUMA) IV: epidemiology of urban malaria in Cotonou (Benin). Malar J 2006;5:45.

60. Berkley JA, Maitland K, Mwangi I, et al. Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study. BMJ 2005;330:995.

61. Gwer S, Newton CRJC, Berkley JA. Over-diagnosis and co-morbidity of severe malaria in African children: a guide for clinicians. Am J Trop Med Hyg 2007;77:6–13.

62. Nnedu ON, Rimel B, Terry C, Jalloh-Vos H, Baryon B, Bausch DG. Syndromic diagnosis of malaria in rural Sierra Leone and proposed additions to the national integrated management of childhood illness guidelines for fever. Am J Trop Med Hyg 2010;82:525–8.

63. Tayler-Smith Katie, Kociejowski Alice, Nadine de Lamotte, Gerard Seco, Ponsar Frederique, Philips Mit ZR. Free treatment, rapid malaria diagnostic tests and malaria village workers can hasten progress toward achieving the malaria related Millennium Development goals: the Médecins Sans Frontières experience from Chad, Sierra Leone and Mali. J Public Heal Africa 2011;2:e1213.

64. Evans JA, Adusei A, Timmann C, et al. High mortality of infant bacteraemia clinically indistinguishable from severe malaria. QJM 2004;97:591–7.

65. FIND 2012 Activity Report [Internet]. [cited 2013 Mar 10];Available from: http://www.finddiagnostics.org/export/sites/default/about/annual_reports/annual_report_2012.pdf

66. Bates I, Maitland K. Are Laboratory Services Coming of Age in Sub-Saharan Africa ? AND DIAGNOSTIC. 2006;42:383–4.

67. Ansumana R. Use of fast technology for the analysis of nucleic acids to store ribonucleic acid extracts at elevated temperatures. Int J Collab Res Intern Medi 2011;3(3):261.

68. Breu F, Guggenbichler S, Wollmann J. World Health Statistics 2013. Vasa [Internet] 2013;168. Available from: http://medcontent.metapress.com/index/A65RM03P4874243N.pdf

69. MOHS SL. NATIONAL PLAN OF ACTION FOR INTERGRATED CONTROL OF ONCHOCERCIAISIS, SCHISTOSOMIAIS, SOIL TRANSMITTED HELMINTHIASIS AND ELIMINATION OF LYMPHATIC FILARIASIS IN SIERRA LEONE. [Internet]. Freetown: Available from: http://files.givewell.org/files/Round2Apps/Cause1/GNNTDC/B/Sierra Leone - Plan of Action for integrated control-final.doc_br

70. Roca T. Human development Report 2013. The Rise of the South, Human Progress in a Diverse World. Afr Contemp 2013;246:164.

71. World Bank. World DataBank. DOI http//databank.worldbank.org/data/home.aspx [Internet] 2013;Date Accessed: 11/01/2013–8/02/2013. Available from: databank.worldbank.org/data/home.aspx

72. World Health Organization. World Health Statistics 2010. 2010.

73. MOHS. National Leprosy and Tuberculosis Control Programme, annual report. Freetown: 2010.

74. World Health Organization, Organization WH. World Health Statistics 2008 [Internet]. 2008. Available from: http://www.who.int/whosis/whostat/EN_WHS08_TOCintro.pdf

75. Winkler MS, Knoblauch AM, Righetti AA, et al. Baseline health conditions in selected communities of northern Sierra Leone as revealed by the health impact assessment of a biofuel project. Int Health [Internet] 2014;6(3):232–41. Available from: http://inthealth.oxfordjournals.org/cgi/doi/10.1093/inthealth/ihu031

76. Knoblauch A, Hodges M, Bah M, et al. Changing Patterns of Health in Communities Impacted by a Bioenergy Project in Northern Sierra Leone. Int J Environ Res Public Health [Internet] 2014;11(12):12997–3016. Available from: http://www.mdpi.com/1660-4601/11/12/12997/

77. Schoepp RJ, Rossi CA, Khan SH, Goba A, Fair JN. Undiagnosed acute viral febrile illnesses, Sierra Leone. Emerg Infect Dis 2014;20(7):1176–82.

78. McConkey SJ. Case series of acute abdominal surgery in rural Sierra Leone. World J Surg 2002;26:509–13.

79. Kebede S, Conteh IN, Steffen CA, et al. Establishing a national influenza sentinel surveillance system in a limited resource setting, experience of Sierra Leone. Health Res Policy Syst [Internet] 2013;11:22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3694480&tool=pmcentrez&rendertype=abstract

80. Torlesse H, Wurie IM, Hodges M. The use of immunochromatography test cards in the diagnosis of hepatitis B surface antigen among pregnant women in West Africa. Br J Biomed Sci 1997;54:256–9.

81. Wurie IM, Wurie AT, Gevao SM. Sero-prevalence of hepatitis B virus among middle to high socio-economic antenatal population in Sierra Leone. West Afr J Med [Internet] 2005 [cited 2014 May 17];24(1):18–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15909704

82. Kouyoumdjian Dr. F, Seisay AL, Kargbo B, Khan SH. Population seeking voluntary HIV counselling and testing in Kenema district, Sierra Leone. XVII Int. AIDS Conf. 3-8 August 2008, Mex. City, Mex. 2008;

83. Homolka S, Post E, Oberhauser B, et al. High genetic diversity among Mycobacterium tuberculosis complex strains from Sierra Leone. BMC Microbiol 2008;8:103.

84. Koroma JB, Bangura MM, Hodges MH, Bah MS, Zhang Y, Bockarie MJ. Lymphatic filariasis mapping by Immunochromatographic Test cards and baseline microfilaria survey prior to mass drug administration in Sierra Leone. Parasit. Vectors. 2012;5:10.

85. Hodges MH, Smith SJ, Fussum D, et al. High coverage of mass drug administration for lymphatic filariasis in rural and non-rural settings in the Western Area, Sierra Leone. Parasit Vectors [Internet] 2010 [cited 2014 Dec 3];3:120. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3018440&tool=pmcentrez&rendertype=abstract

86. Koroma JB, Sesay S, Sonnie M, et al. Impact of Three Rounds of Mass Drug Administration on Lymphatic Filariasis in Areas Previously Treated for Onchocerciasis in Sierra Leone. PLoS Negl Trop Dis 2013;7.

87. Hodges ME, Koroma JB, Sonnie M, Kennedy N, Cotter E, Macarthur C. Neglected tropical disease control in post-war Sierra Leone using the Onchocerciasis Control Programme as a platform. Int. Health. 2011;3:69–74.

88. Fichet-Calvet E, Rogers DJ. Risk maps of lassa fever in West Africa. PLoS Negl Trop Dis 2009;3.

89. Pezzoli L, Conteh I, Kamara W, et al. Intervene before leaving: clustered lot quality assurance sampling to monitor vaccination coverage at health district level before the end of a yellow fever and measles vaccination campaign in Sierra Leone in 2009. BMC Public Health. 2012;12(1):415.

90. Ansumana R, Malanoski AP, Bockarie AS, et al. Enabling methods for community health mapping in developing countries. Int J Health Geogr 2010;9:56.

91. Leski TA, Lin B, Malanoski AP, et al. Testing and validation of high density resequencing microarray for broad range biothreat agents detection. PLoS One 2009;4.

92. Mark Porit, Lindsay Meyers, Amber Lewis, Kody Nilsson, Paul Murphy, Meghan Hamilton , Mike Vaughn, Stephanie Thatcher, Jamey Hulsberg, Ron Mudrow, Ben Smith JF, David Jones, Cory Estes, David Teng, Rob Crisp, Rich Abbott, Steven Dobrowolski, Anne Blaschke, Kent Korgenski, Judy Daly Carrie Byington KR. Analysis of 250 Pediatric NPA Samples for 21 Respiratory Pathogens Using an Automated, Nested Multiplex PCR Platform [Internet]. [cited 2014 Jun 1];Available from: http://www.biofiredx.com/pdfs/Posters/2008/IdahoTechnology_FilmArray_PASCV2008Poster.pdf

93. Poritz MA, Blaschke AJ, Byington CL, et al. Filmarray, an automated nested multiplex PCR system for multi-pathogen detection: Development and application to respiratory tract infection. PLoS One 2011;6.

94. Tayeb LA, Lefevre M, Passet V, Diancourt L, Brisse S, Grimont PAD. Comparative phylogenies of Burkholderia, Ralstonia, Comamonas, Brevundimonas and related organisms derived from rpoB, gyrB and rrs gene sequences. Res Microbiol 2008;159:169–77.

95. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 2007;73:5261–7.

96. Leski TA, Bangura U, Jimmy DH, et al. Multidrug-resistant tet(X)-containing hospital isolates in Sierra Leone. Int J Antimicrob Agents 2013;42:83–6.

CHAPTER TWO

COMMON INFECTIONS IN SIERRA LEONE: PRESUMPTIVE SELF-DIAGNOSIS OF MALARIA AND OTHER FEBRILE ILLNESSES IN SIERRA LEONE

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Keywords: *Malaria, fevers, self-care, health services accessibility, community pharmacy services, West Africa*

2.1 ABSTRACT

Background: The objective of this study was to evaluate the prevalence of self-diagnosis of malaria and other febrile illnesses in Bo, Sierra Leone.

Methods: All households in two neighboring sections of Bo were invited to participate in a cross-sectional survey.

Results: A total of 882 households (an 85% participation rate) that were home to 5410 individuals participated in the study. Of the 910 individuals reported to have had what the household considered to be malaria in the past month, only 41% were diagnosed by a healthcare professional or a laboratory test. Of the 1402 individuals reported to have had any type of febrile illness within the past six months, only 34% had sought a clinical or laboratory diagnosis. Self-diagnosis of influenza, yellow fever, typhoid, and pneumonia was also common.

Conclusion: Self-diagnosis and presumptive treatment with antimalarial drugs and other antibiotic medications that are readily available without a prescription may compromise health outcomes for febrile adults and children.

2.2 BACKGROUND

The overuse or inappropriate use of antimalarial medications and antibiotics is a growing concern in many parts of the world, including Sierra Leone, a post-conflict country in West Africa. In Sierra Leone, these drugs are dispensed by government-run clinics and hospitals, private healthcare facilities, licensed pharmacies, and through the informal sectors[1]. Because medications are readily available without a prescription, people with self-diagnosed infections can access treatments without first seeking a formal clinical consultation and/or laboratory-confirmed diagnosis. A survey conducted in April 2009 in several parts of Sierra Leone—including the city of Bo, the focus of this paper—found that 50.8% of anti-malarial drugs were dispensed without a prescription [1].

Easy access to antimalarial drugs and other antibiotic agents may compromise patient health outcomes. If the medications purchased for an infection are inappropriate—such as antibiotics taken for a viral infection or chloroquine taken for a chloroquine-resistant case of malaria—or if medications are not taken in the appropriate dosage and for an appropriate length of time, self-diagnosis and treatment may contribute to prolonged illness, more severe morbidity, and an increased risk of mortality. A study in Bo district in 2008 found that only 48.3% of malaria-positive patients at a hospital that offered free care completed the full course of prescribed antimalarial treatment [2]. Given that low rate of adherence to prescribed treatment regimens, it is likely that few patients who access over-the-counter antibiotics complete a full course of an appropriate, government-recommended formulation. The antimalarial medications available over-the-counter are rarely the formulations recommended by the national government and the World Health Organization (perhaps a good sign that national health system drugs are not leaking into the private market), and the 2009 study found a very low level of knowledge about national and international antimalarial policies and regulations among private-sector sellers of medication, compared to a high level of knowledge among public-sector providers [1].

Furthermore, misuse of antimicrobial agents can contribute to the emergence of drug resistance, which is a growing concern in Sierra Leone. A 2002-2003 study found treatment failure among more than half of pediatric malaria patients who tested positive by PCR for malaria and treated with chloroquine[3]. Treatment failure was assessed by PCR after treatment

and was also frequently observed for sulphadoxine-pyrimethamine (SP) [3]. These results suggest that drug-resistant malaria is already common in the study area. Evidence of drug-resistant bacterial infections has also been reported, including outbreaks of multidrug-resistant *Shigella dysenteriae* in 1999-2000 [4] and drug resistant *Staphylococcus aureus* among children during a 2008-2009 study in Freetown [5]. Sierra Leone has one of the highest rates of multidrug-resistant tuberculosis (MDR-TB) in sub-Saharan Africa [6].

Over-diagnosis of malaria, in particular, may be common. Even clinicians can find it difficult to diagnose malaria accurately solely based on symptoms. A study in the Bo district in 2005 found that 82% of children suspected by clinical officers to have malaria based on physical symptoms such as fever, splenomegaly, and vomiting tested positive for parasitemia by a Paracheck-pf ® rapid diagnostic test (RDT) and 18% did not [7]. Between 2004 and 2006 in the same region of Sierra Leone, only 65% of the Paracheck® RDTs performed on pediatric patients with clinically-suspected malaria were positive, suggesting that antimalarial medication may have been significantly over-prescribed without the use of confirmatory laboratory tests [8]. It is important to note that some children in highly-endemic areas test negative for malaria at the beginning of a febrile illness, such as a case of pneumonia, but then become malaria-positive later in their course of illness due to their weakened state. (Additionally, many children test positive for malaria even when they are asymptomatic.) Parents without clinical training might be more likely than healthcare professionals to diagnose any febrile illness as malaria and to seek presumptive antimalarial treatment. While presumptive treatment may be helpful—and perhaps even lifesaving—when the child actually does have malaria, a misdiagnosis may result in delayed treatment for the actual cause of the fever, and the delay in seeking professional medical care may increase morbidity and mortality [9].

The goal of this paper was to evaluate the prevalence of self-diagnosis of malaria and other febrile illnesses in Bo, Sierra Leone's second largest city.

2.3 METHODS

2.3.1 Sampling Strategy.

All households within the Kulanda Town and Njai Town sections (neighborhoods) of the city of Bo were eligible for participation. The research laboratory has previously mapped all of the buildings within these sections and conducted a household census, in 2010, to identify which structures were residential ones [10]. The resulting geographic information system (GIS) was used to create a map of all of the homes within the study community. In June 2012, members of the research team visited each household on the map, as well as 11 new households (which were added to an updated map), to ask for their participation. Of the 1038 households in these sections, 882 (85.0%) agreed to participate. For each of these households, one adult, usually the head of the household, was interviewed to gather information about the whole household.

2.3.2 Data Collection.

Each interview began with questions about the household's environmental characteristics, such as building materials and access to utilities, and about household demographics, such as the age and sex of each current resident. Students at boarding school, adults working in another town and not sleeping at the residence in Bo, and others who spent at least 6 months of the past year living elsewhere were not considered to be current household members.

Then a series of questions were asked about febrile illnesses experienced by household members. These questions were developed in consultation with residents of the Kulanda Town / Njai Town sections to ensure clarity, and asked about all household members of all ages. First, the household representative was asked whether anyone currently living in the household had been ill with what the household considered to be malaria in the past one month. If malaria was reported to have occurred, follow-up questions asked where those with malaria were diagnosed (such as at a hospital or clinic, at home by a nurse, or at home by an untrained person—that is, self-diagnosis) and whether they were tested for malaria by

a laboratory. A second set of questions asked whether anyone currently living in the household had a febrile illness earlier in 2012 (that is, in the 6 months prior to the interview). Follow-up questions asked about the frequency and duration of febrile illnesses; symptoms associated with these fevers (such as joint tenderness, headaches, altered behavior, and jaundice); and whether the febrile person was examined by a clinician and/or had a laboratory test to determine the cause of the fever. A final set of yes/no questions asked whether the household considered anyone currently living in the household to have ever had any of more than a dozen listed communicable and non-communicable conditions, and whether those diagnoses were made by a doctor or were self-diagnosed.

2.3.3 Data Management and Analysis.

Responses were entered by the interviewers directly into a Filemaker Pro 12 relational database on a password-protected tablet computer. Households were identified by a number linked to a map stored at the research laboratory; these codes were random and not related to the geographic coordinates of the map. Data were analyzed using the statistical software program SPSS (version 20). Proportions, means, and standard deviations were used to describe the variables. Chi-squared tests were used to compare rates in independent populations, such as different age groups.

2.3.4 Ethical Considerations.

Adults ages 18 and older were interviewed after providing informed consent, which was documented with a signature or a thumbprint. The consent form and study materials were available in both English and Krio, the local language in Sierra Leone. No compensation or other incentive was offered. To protect the confidentiality of information shared with the research team, no names or addresses were entered into the database. The data entered into each tablet computer were deleted daily after the data files on the tablets were downloaded to a password-protected desktop computer in a locked and guarded research facility. The research protocol was approved by the Sierra Leone Ethics and Scientific Review Committee and by Njala University (Sierra Leone), the Liverpool School of Tropical Medicine (UK), the U.S. Naval Research Laboratory (USA), and George Mason University (USA).

2.4 RESULTS

The 882 participating households contained 5410 individuals, with a mean household size of 6.1 persons. The households reported a somewhat diverse set of socio-environmental characteristics. While 1186 (66.7%) of the 1778 beds reported to be located in the participating homes were said to have bednets, 328 (37.2%) of households reported having no bednets. In total, 877 (99.4%) of households reported seeing a rat in the house in the past month, 860 (97.5%) reported seeing cockroaches in the house in the past month, 677 (76.8%) had a tile or concrete rather than dirt floor, 546 (61.9%) had a trash bin in the home (of which 153 covered the bin), 353 (40.0%) had electricity, and 155 (17.6%) had a drinking water source within 50 meters of the home.

In total, 675 (76.5%) of the 882 households reported at least one case of malaria (as defined by the household) in the month prior to the survey, with a total of 910 (16.8%) of the 5410 individuals reported to have had malaria during that time period. However, 540 (59.3%) of these 910 individuals were presumptively diagnosed by the ill person or a household member, and only 370 (40.7%) were diagnosed following laboratory testing.

A total of 1402 (25.9%) of the individuals within participating households were reported to have had any type of febrile illness (whether caused by malaria or another condition) within the past six months. The rate of fever reported differed by age group and by sex, with young children (those 0 to 4 years old) having the highest rate ($p<0.001$) and females reporting more fevers than males ($p<0.001$) (**Table 2.1**). Only 33.9% of people with fever were reported to have had laboratory tests to determine the cause of the fever. There were significant differences in the likelihood of testing by age ($p=0.011$). Households with indicators of higher socioeconomic status (SES), such as those with electricity in the home or a drinking water source very near to the home, generally reported slightly lower rates of febrile illness within the household (**Table 2.2**). Markers of household SES were not significantly associated with reported testing rates.

Self-diagnosis of several other conditions was common (**Table 2.3**). All of the 234 individuals reported to have ever had influenza, as defined by the household, indicated a

self-diagnosis. More than 96% of the 160 persons reported to have had what the household considered to be yellow fever (which had been the focus of a recent vaccination campaign [11]) reported self-diagnosis, as did more than 60% of the 445 persons reported to have had typhoid (which is a relatively common laboratory diagnosis in Bo, as per Widal tests). More than half of the 317 people reported to have had what the household designated as pneumonia were self-diagnosed. However, diagnosis of less common and more specific conditions, such as bacterial meningitis, hepatitis B, and hepatitis C, were nearly always reported to have been diagnosed by a doctor and not self-diagnosed by the household.

Table 2.1: Prevalence of reported febrile illnesses and testing in the past 6 months, by age and sex

Table 2.1: Prevalence of reported febrile illnesses and testing in the past 6 months, by age and sex

Age Group	**Total**			**Females**			**Males**			**p-value for Chi-squared test of difference by sex (2-tailed)**	
	n	n (%) reported to have had fever	Of those reported to have had a fever, n (%) who had tests to determine the cause of the fever	n	n (%) reported to have had fever	Of those reported to have had fever, n (%) tested	n	n (%) reported to have had fever	Of those reported to have had fever, n (%) tested	reported fever cases	testing for cause among those reported to have had fever
0-4	705	368 (52.2%)	136 (37.0%)	348	193 (55.5%)	72 (37.3%)	357	175 (49.0%)	64 (36.6%)	0.088	0.885
5-14	1527	423 (27.7%)	128 (30.3%)	835	233 (27.9%)	77 (33.0%)	692	190 (27.5%)	51 (26.8%)	0.847	0.169
15-29	1739	293 (16.8%)	99 (33.8%)	973	181 (18.6%)	53 (29.3%)	766	112 (14.6%)	46 (41.1%)	0.027	0.040
30-44	803	136 (16.9%)	58 (42.6%)	423	86 (20.3%)	30 (34.9%)	380	50 (13.2%)	28 (56.0%)	0.003	0.018
45-59	381	95 (24.9%)	36 (37.9%)	180	56 (31.1%)	19 (33.9%)	201	39 (19.4%)	17 (43.6%)	0.009	0.351
≥60	255	87 (34.1%)	19 (21.8%)	134	52 (38.8%)	12 (23.1%)	121	35 (28.9%)	7 (20.0%)	0.090	0.749
Total	5410	1402 (25.9%)	476 (34.0%)	2893	801 (27.7%)	263 (32.8%)	2517	601 (23.9%)	213 (35.4%)	0.001	0.309

1402 (25.9%) of household members reported fever, of whom 476 (34.0%) were tested to determine the cause of the fever. In total, 801 (27.7%) females and 601 (23.9%) males reported fever, and 263 (32.8%) of females and 213 (35.4%) of males with fever were tested. The rate of fevers reported was different by sex and by age group, but there were not significant age and sex differences in testing.

Table 2.2: Household environmental characteristics and reported febrile illnesses in the household (HH)

Table 2.2: Household environmental characteristics and reported febrile illnesses in the household (HH)

Household (HH) feature (among 882 households)	1+ HH member reported to have had malaria in the past month			Of those HHs with malaria, 1+ person with malaria reported to have had malaria testing			1+ HH member reported to have had any febrile illness in the past 6 months			Of those HHs reporting fever, 1+ person with fever reported to have had a formal diagnosis		
	among HHs with this feature	**among HHs without this feature**	**p- value**	**among HHs with this feature**	**among HHs without this feature**	**p- value**	**among HHs with this feature**	**among HHs without this feature**	**p- value**	**among HHs with this feature**	**among HHs without this feature**	**p- value**
Fewer than six individuals in the household (n=552, 62.8% of HHs)	417 (75.3%)	258 (78.7%)	0.252	125 (30.0%)	102 (39.5%)	0.011	453 (81.8%)	269 (82.0%)	0.932	129 (28.5%)	172 (63.9%)	<0.001
Households with at least one bednet (n=691, 78.3%)	517 (74.8%)	158 (82.7%)	0.020	176 (34.0%)	51 (32.3%)	0.686	584 (84.5%)	138 (72.3%)	<0.001	248 (42.5%)	53 (38.4%)	0.387
Having a tile or concrete floor rather than a dirt floor (n=677, 76.8%)	508 (75.0%)	167 (81.5%)	0.055	177 (34.8%)	46 (27.5%)	0.081	539 (79.6%)	183 (89.3%)	0.001	217 (40.3%)	84 (45.9%)	0.183
Having a trash bin in the home (n=546, 61.9%)	388 (71.1%)	287 (85.4%)	<0.001	138 (35.6%)	85 (29.6%)	0.105	428 (78.4%)	294 (87.5%)	0.001	163 (38.1%)	138 (46.9%)	0.018
Having electricity (n=353, 40.0%)	264 (74.8%)	411 (77.7%)	0.320	90 (34.1%)	133 (32.4%)	0.641	271 (76.8%)	451 (85.3%)	0.002	112 (41.3%)	189 (41.9%)	0.880
Using a drinking water source within 50 meters of the home (n=155, 17.6%)	106 (68.4%)	569 (78.3%)	0.010	27 (25.5%)	196 (34.4%)	0.070	124 (80.0%)	598 (82.3%)	0.505	52 (41.9%)	249 (41.6%)	0.949

Malaria and febrile illnesses were significantly more likely to be reported by households without at least one bednet, those with a dirt floor, those without a trash bin in the home, those without electricity, and those without a drinking water source near the home. Testing of at least one household member was less likely to be reported by smaller households and those without a trash bin in the home.

Table 2.3: Reports of family medical history, by source of diagnosis

Table 3.3: Reports of family medical history, by source of diagnosis			
Condition	n (%) of households reporting that someone currently living in the household has **ever had this condition**	Of households reporting this condition, n (%) reporting that the condition was **diagnosed by a doctor**	Of households reporting this condition, n (%) reporting that the condition was **self-diagnosed**
Influenza	234 (26.5%)	0 (0%)	234 (100%)
Lassa fever	5 (0.6%)	0 (0.0%)	5 (100%)
Yellow fever	160 (18.1%)	6 (3.8%)	154 (96.3%)
Common cold	774 (87.8%)	180 (23.3%)	594 (76.7%)
Asthma	79 (9.0%)	21 (26.6%)	58 (73.4%)
Typhoid fever	445 (50.5%)	177 (39.8%)	268 (60.2%)
Pneumonia	317 (35.9%)	151 (47.6%)	166 (52.4%)
Bacterial meningitis	10 (1.1%)	8 (80.0%)	2 (20.0%)
Hepatitis B	5 (0.6%)	5 (100%)	0 (0.0%)
Hepatitis C	1 (0.1%)	1 (100%)	0 (0.0%)
Tuberculosis	0 (0.0%)	0 (0.0%)	0 (0.0%)
Rift Valley fever	0 (0.0%)	0 (0.0%)	0 (0.0%)
Dengue fever	0 (0.0%)	0 (0.0%)	0 (0.0%)
Households reported a high rate of self-diagnosis for conditions such as influenza (100% of reported cases self-diagnosed), yellow fever (96.3% self-diagnosed), colds (76.7% self-diagnosed), typhoid fever (60.2% self-diagnosed), and pneumonia (52.4% self-diagnosed).			

2.5 DISCUSSION

We found that the majority of febrile illnesses in Bo, Southern Province, Sierra Leone, are self-diagnosed without clinical examination or laboratory testing, including the more than half of suspected malaria cases that are treated presumptively without any clinical diagnostics. The fact that households with greater numbers of individuals were more likely to report that at least one household member had been tested for a febrile illness supports the validity of reports about diagnosis and testing rates, since a greater number of persons in the household increases the probability of a severe illness occurring for at least one resident. The validity of the survey instrument is also supported by the low numbers of households reporting uncommon diseases such as bacterial meningitis unless these conditions were diagnosed by a doctor.

These results are similar to those from studies in other parts of West Africa which have found that more than half of adults self-diagnose fevers and self-medicate for what they consider to be malaria [12,13]. Of those who self-treat, only a small proportion know the correct dosage for common antimalarial medications [12].

This high rate of self-treatment is concerning, since it is likely that a significant proportion of these presumptive cases are treated inappropriately (as per the introduction to this paper). Some people who would benefit from antibiotic and supportive therapy may not be receiving adequate care, and many people who purchase medication for their self-diagnosed malaria or other conditions may be taking drugs that are ineffective for their condition. Febrile individuals who self-treat may experience disease complications and increased treatment costs resulting from delayed access to appropriate medications and other therapy [14]. Taking the wrong medication, such as taking antimalarials for a fever caused by a different infectious agent, generally means that the actual infection remains untreated for at least several days.

When tests are available at a reasonable cost, testing before treating may significantly reduce the inappropriate use of antimicrobial medications. For example, a cohort study of children ages 1 to 10 years in Uganda found that only 32% of fevers were caused by malaria, and the researchers concluded that a test-before-treating approach in that study population would reduce the use of antiparasitic drugs by two-thirds [15]. The reduction of unnecessary treatment

achieved by a test-then-treat approach will, by definition, be lower in higher-endemicity areas, including parts of Sierra Leone, where a recent community-based study in the Bo area found that 83% of febrile women and young children who were tested had malaria [16]. Even so, testing may significantly reduce the number of people taking unnecessary medications. The 17% of the 17,130 tested individuals who were malaria-negative in the Bo area study represented nearly 3000 people who avoided the unnecessary use of antimalarial drugs [16].

Testing does not have to occur in a clinical setting. In rural Sierra Leone, free malaria testing by community health workers (CHWs) has increased access to diagnosis for thousands of households [16]. Those who test positive for malaria are treated by the CHWs, and those with negative malaria tests or complicated malaria cases are referred to a nearby hospital for advanced care (although the follow-up rate for referrals from this program has been shown to be very low). In some places, testing and treatment by CHWs has been found to be preferred over home treatment [17]. However, one potential challenge to testing programs is convincing healthcare providers and patients not to prescribe or take antimicrobials after a negative test result. A study from Ghana found that more than half of patients who tested negative for malaria were prescribed antimalarials anyway [18], and some studies from other parts of Africa have found similar results [19]. Taking antimalarial medication "just in case" may be seen as the best option when households do not have the resources to travel to and pay for further clinical examination and testing.

Besides the direct benefits to patients, expanded use of testing before treatment may slow the further development of drug resistance by reducing the proportion of the population accessing pharmaceutical agents without prescriptions tailored to their actual diagnoses. Patients who have seen a clinician and have a confirmed diagnosis may be more likely than others to complete a full course of an appropriate antibiotic or antimalarial, especially if their treating clinicians counsel them about the importance of compliance with prescribed treatments (an occurrence dependent on those practitioners having the time and resources to provide health education). Community-based behavior change communication processes may also help to promote healthy use of pharmaceuticals by households and communities, but when these public health structures are not in place the burden of health education typically falls on clinicians.

2.6 LIMITATIONS.

This study had several limitations that require a conservative interpretation of the findings. No laboratory tests were conducted to confirm the reported causative agents for participants' febrile illnesses, so we do not know how often their self-diagnoses were accurate. Additionally, participants were not asked about their use of antibiotic and antiparasitic drugs, such as what medications they preferred to take when febrile or where they procured these medications. Because these questions were not part of the survey, we have limited information about whether the self-treatment used by febrile participants was appropriate for their illnesses.

2.7 CONCLUSIONS.

This study provides evidence that self-diagnosis and self-medication for malaria and other febrile illnesses is common in Bo, Sierra Leone. In order to better understand the implications of self-diagnosis and presumptive treatment on patient health outcomes, we recommend that further studies evaluate the types of infections common in this population to see how well clinical laboratory results match self-diagnoses. Future research should also examine the pharmaceutical access and use habits of local residents to see whether appropriate courses of medication are being taken by those with and without prescriptions from a clinician. Understanding the knowledge, attitudes and beliefs, and health practices and behaviors of residents regarding diagnosis and treatment of fevers may contribute to improved health services, policies, and practices.

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Contribution of Authors

RA, KHJ, AAG, MHH, TAL, APM, BL, MJB, and DAS were involved in the conception and design of the study. RA and JML collected the data. RA and KHJ conducted data analysis and drafted the manuscript. All authors critically reviewed the manuscript and approved its submission.

2.8 REFERENCES

1. Diap G, Amuasi J, Boakye I, Sevcsik A-M, Pecoul B. Anti-malarial market and policy surveys in sub-Saharan Africa. Malar J 2010;9 Suppl 1:S1.

2. Gerstl S, Dunkley S, Mukhtar A, Baker S, Maikere J. Successful introduction of artesunate combination therapy is not enough to fight malaria: Results from an adherence study in Sierra Leone. Trans R Soc Trop Med Hyg 2010;104:328–35.

3. Checchi F, Roddy P, Kamara S, et al. Evidence basis for antimalarial policy change in Sierra Leone: Five in vivo efficacy studies of chloroquine, sulphadoxine-pyrimethamine and amodiaquine. Trop Med Int Heal 2005;10:146–53.

4. Guerin PJ, Brasher C, Baron E, et al. Case management of a multidrug-resistant Shigella dysenteriae serotype 1 outbreak in a crisis context in Sierra Leone, 1999-2000. Trans R Soc Trop Med Hyg 2004;98:635–43.

5. Foday S, Adesida SA, Christian H, V KT, Thomashire A, Doris H. Sierra Leone Journal of Biomedical Research Original Paper Nasal Carriage of Staphylococcus aureus and Antibiotic Susceptibility Pattern among Children in Freetown , Sierra Leone Demographic Profile of Study Population. 2010;2(1):65–9. Available from: http://ajol.info/index.php/sljbr/index

6. Zignol M, Hosseini MS, Wright A, et al. Global incidence of multidrug-resistant tuberculosis. J Infect Dis 2006;194:479–85.

7. Nnedu ON, Rimel B, Terry C, Jalloh-Vos H, Baryon B, Bausch DG. Syndromic diagnosis of malaria in rural Sierra Leone and proposed additions to the national integrated management of childhood illness guidelines for fever. Am J Trop Med Hyg 2010;82:525–8.

8. Tayler-Smith Katie, Kociejowski Alice, Nadine de Lamotte, Gerard Seco, Ponsar Frederique, Philips Mit ZR. Free treatment, rapid malaria diagnostic tests and malaria village workers can hasten progress toward achieving the malaria related Millennium Development

goals: the Médecins Sans Frontières experience from Chad, Sierra Leone and Mali. J Public Heal Africa 2011;2:e1213.

9. Hopkins H, Asiimwe C BD. Access to antimalarial therapy: accurate diagnosis is essential to achieving long term goals. BMJ 2009;339:b2606.

10. Ansumana R, Malanoski AP, Bockarie AS, et al. Enabling methods for community health mapping in developing countries. Int J Health Geogr 2010;9:56.

11. Pezzoli L, Conteh I, Kamara W, et al. Intervene before leaving: clustered lot quality assurance sampling to monitor vaccination coverage at health district level before the end of a yellow fever and measles vaccination campaign in Sierra Leone in 2009. BMC Public Health [Internet] 2012 [cited 2014 May 17];12(1):415. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3438100&tool=pmcentrez&rendertype=abstract

12. Nsagha D, Njunda A, Kamga H, et al. Knowledge and practices relating to malaria in a semi-urban area of Cameroon: choices and sources of antimalarials, self-treatment and resistance. Pan Afr. Med. J. 2011;9.

13. Uzochukwu BS, Onwujekwe OE. Socio-economic differences and health seeking behaviour for the diagnosis and treatment of malaria: a case study of four local government areas operating the Bamako initiative programme in south-east Nigeria. Int J Equity Health [Internet] 2004 [cited 2014 May 23];3(1):6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=544024&tool=pmcentrez&rendertype=abstract

14. Amexo M, Tolhurst R, Barnish G, Bates I. Malaria misdiagnosis: Effects on the poor and vulnerable. Lancet. 2004;364:1896–8.

15. Njama-Meya D, Clark TD, Nzarubara B, Staedke S, Kamya MR, Dorsey G. Treatment of malaria restricted to laboratory-confirmed cases: a prospective cohort study in Ugandan children. 2007.

16. Thomson A, Khogali M, de Smet M, et al. Low referral completion of rapid diagnostic test-negative patients in community-based treatment of malaria in Sierra Leone. Malar J 2011;10:94.

17. Onwujekwe O, Dike N, Ojukwu J, et al. Consumers stated and revealed preferences for community health workers and other strategies for the provision of timely and appropriate treatment of malaria in southeast Nigeria. Malar J 2006;5:117.

18. Ansah EK, Narh-Bana S, Epokor M, et al. Rapid testing for malaria in settings where microscopy is available and peripheral clinics where only presumptive treatment is available: a randomised controlled trial in Ghana. BMJ 2010;340:c930.

19. Graz B, Willcox M, Szeless T, Rougemont A. “Test and treat” or presumptive treatment for malaria in high transmission situations? A reflection on the latest WHO guidelines. Malar J 2011;10:136.

COMMON NON-MALARIAL FEBRILE ILLNESSES IN SIERRA LEONE: VIRAL HEPATITIS A, B AND C INFECTIONS

3.1 ABSTRACT

Background

Viral hepatitis is a common and sometimes serious infectious disease. Globally, over 400 million people are chronically infected by the hepatitis B virus[3]; over 170 million people affected by hepatitis C virus [4] and tens of millions affected by hepatitis A.[5] Many developing countries such as Sierra Leone, are characterized by significant health and hygiene challenges that predispose to the transmission of hepatitis viruses.

Objectives

The goal of this research was to investigate the prevalence and distribution of viral hepatitis in Bo, Sierra Leone.

Methods

A cohort of 1403 persons was followed for one year starting on July 7th, 2012 to July 7th, 2013. Blood samples were collected from febrile subjects and about 0.5-1.5mls of blood were used in analyzes and 0.5-1ml for storage and/ further immunochromatographic test, (ICT) or lateral flow assay (LFA) analysis as required. . About 5 -100µl of blood were used per ICT. The following rapid tests were used: Hepatitis, HBsAg and Hepatitis Combo (HBsAg, HBeAg, HBsAb HBeAb, HBcAb), HCV and HAV IgG/IgM (Standard Diagnostics, Seoul, Korea). All tests were uploaded via a portable, battery-operated LF assay reader/imager (Deki reader, Fio Corporation, Toronto, Canada) to a cloud database at www.fio.net.com for a quality assessment.

Results

There was an average monthly attendance of 160.25 SD±10.63 for the twelve months of cohort study, with a significant difference in monthly attendances ($P<0.001$). A monthly average of 10.4±1.9SD for Hepatitis B; 5.5±2.5SD for Hepatitis C and 8.9(±3.4SD) for Anti-Hepatitis A were recorded. The prevalence of hepatitis B was 9.7% and hepatitis C, 4.7% while seroprevalence of Hepatitis A was 8.7%. Two age groups, 15-29 years, and ≥ 45years had higher rates of both HCV and HBV while more females were infected than males.

Syndromically, 32% fever, 22% cough, 15% headache and 16% abdominal pain were the commonest syndromes of viral hepatitis among male subjects. In female subjects, fever (32%), headache (18%), abdominal pain (18%), cough (15%) were the commonest syndromes. However, these syndromes are not unique to viral hepatitis and may not be precise.

The average specific gravity was 1.94±0.85SE. The normal range for specific gravity of urine is 1.003-1.020[17], this high average range is indicative of high urine concentration or dehydration of subjects. About 50.04% (95% CI 47.21% -52.85%) were cloudy; 10.21 %(95%CI 8.63-12.04%) had proteinuria and 57.7 %(95%CI: 54.9%-60.45%) had traces of protein in their urine. Though dark or cloudy urine could mean infections of other etiologies, viral hepatitis has been associated with dark urine[18] and protienuria[19].

Conclusion

For every one person affected by hepatitis C in Bo, Sierra Leone, two individuals are affected by Hepatitis B, with no risk of infection from Hepatitis A. The lack of new cases of hepatitis A within the cohort demonstrates a better environmental health within the catchment population of Mercy Hospital. Notwithstanding, viral hepatitis remains a major contributor to illnesses and death in resource-poor countries such as Sierra Leone. The treatment options in Sierra Leone are fewer and hard-to-get for infected persons.

3.2 INTRODUCTION

Viral hepatitis is a common and sometimes serious infectious disease marked by necrosis and inflammation of the liver[1] and contributes to a significant morbidity and mortality worldwide[2]. Hepatitis A viruses are transmitted enterally and lead to usually self-limited acute hepatitis. Viral Hepatitis B (HBV) and Hepatitis C (HCV) are transmitted by parenteral routes and can result in chronic hepatitis, progressing to liver cirrhosis and hepatocellular carcinoma [2].

Globally, over 400 million people are chronically infected by the hepatitis B virus[3]; over 170 million people affected by hepatitis C virus [4] and tens of millions affected by hepatitis A[5]. Every year, over 300,000 cases of liver cancer and similar numbers of gastrointestinal hemorrhage and ascites are due to hepatitis B[3]. Over 100,000 cases of liver cancer per year, with digestive hemorrhage and ascites episodes, are due to hepatitis C virus[4] while hepatitis A remains a major disease of poverty and endemic in resource-poor areas[5].

Many developing countries such as Sierra Leone, are characterized by significant health and hygiene challenges that predispose to the transmission of hepatitis viruses. There is late detection of viral hepatitis for several reasons. Poverty, inadequate prevention and screening services[6], ignorance, lack of easy accessibility to healthcare centers and lack of trained personnel and diagnostic facilities are major reasons. Additionally, un-affordability or lack of expensive drugs, such as Lamivudine, and consultations with quacks and traditional healers compound the problem.[7] The studies of viral hepatitis in Sierra Leone have reported seroprevalences ranging from 5 to 48%, with the most recent one on hepatitis B done in Bo, Sierra Leone[8]. Infrequent studies have been done in the past decade, but all the studies document a high seroprevalence of hepatitis amongst different population groups in Sierra Leone[9] [10]

HBV is of a greater global importance than many other viral causes of human hepatitis[11]. Knowledge of Hepatitis B as an important cause of morbidity and mortality led to a worldwide effort to reduce transmission through routine infant vaccination.[12] Usually, HBV occurs early in life in poor countries.[13] It is transmitted via body fluids vertically (i.e. from mother-to-child), horizontally (i.e. child-to-child), sexually or through parenteral routes[13,14]. Since all modes of transmission are possible in Africa, coupled with resource poverty and weak healthcare systems, the risk of contracting HBV is higher in this region.

There are vaccines against HBV, but Sierra Leone is yet to implement mass vaccinations that target both children and adults. The health sector is yet to couple vaccinations with routine screenings for all viral hepatitis including HBV[9] outside blood donors who are normally healthy persons over 12 years of age. There is a scarcity of data on the disease incidence or prevalence locally.

The goal of this study was to investigate the seroprevalence and distribution of viral hepatitis in Bo Sierra Leone.

3.3 METHODS

3.3.1 Sampling Strategy

The study was conducted at the Mercy Hospital in Bo Sierra Leone. Within the catchment area of the study hospital, 100% of heads of households (about 1027) were interviewed. These houses were identified using an existing digital, interactive map. Each dwelling house was given a unique identifier (NF001 for the first house and NF1000 for the 1000th house). The unique ID given to a house was used for a household (when a single family lives in a house). For houses with multiple households, (families), alphabets A-Z were added to the label (NF001A, NF001B, NF001C; for three households in one house). The interviews were conducted using survey forms entered into FilemakerGo database on Apple Ipads.

A prospective cohort of 15% of households, about 1403 individuals, were randomly selected. To randomly select cohort households, shape files of the catchment area with digitized building footprints were displayed in a Quantum GIS (QGIS) window. Using the Random Selection item of the research tool menu in QGIS, the number of houses were randomly selected and saved as a separate layer. This layer was displayed, and the houses labeled according to their unique code assigned to them ;(e.g. NFC001, NFC002, and so on). The labeled map was then printed out, and the houses with their addresses attached to each code were sampled.

A cohort of 1403 persons was followed for one year starting on July 7^{th,} 2012 to July 7th, 2013. Additional non-cohort data was collected from January 2014 to July 2014 .

3.3.2 Data Collection

3.3.2.1 Immunoassays

Blood samples were collected from febrile subjects and about 0.5-1.5mls of blood were used in analyzes and 0.5-1ml for storage and/ further immunochromatographic test, (ICT) or lateral flow assay (LFA) analysis as required. About 5 -100µl of blood were used per ICT. The following rapid tests were used: Hepatitis, HBsAg and Hepatitis Combo (HBsAg, HBeAg, HBsAb HBeAb, HBcAb), HCV and HAV IgG/IgM (Standard Diagnostics, Seoul, Korea). All tests were uploaded via a portable, battery-operated LF assay reader/imager (Deki reader, Fio Corporation, Toronto, Canada) to a cloud database at www.fio.net.com for a quality assessment (Fig 3.1). The automated reader has been used variously to confirm rapid lateral assays with congruent results[15,16].

3.3.2.2 Syndromic Data Collection

Syndromic data were synchronized with the surveillance system. The system was comprised of a Filmmakers 12 Server hosted on a 19" MacBook Computer with a local area network using a Cisco router and outpatient records digitized on the network (Fig 3.1).

Figure 3.1: Syndromic Data Collection and Device-to-cloud system

3.3.2.3 Urinalyses

Urine samples collected were analyzed for parameters such as color, appearance, specific gravity, and contents, using the urine dipstick, Multistix® 10SG (Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA). The samples were centrifuged, and microscopic smear ensued alongside with the Bayer Atlas of Urine Sediments (Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA).

3.3.3 Ethics

Ethical approval was sought in five institutions: the Liverpool School of Tropical Medicine (LSTM), the Njala University Sierra Leone (NU), Sierra Leone Ethics and Scientific Review Committee, George Mason University (GMU) and the US Naval Research Laboratory (NRL). Study site, Mercy Hospital Research Laboratory, has a joint research agreement that ensures all protocols are reviewed by the George Mason University Review Board, Njala University Institutional Review Board, and Naval Research Laboratory Ethics Board. The

Sierra Leone Ethics and Scientific Review Committee of the Sierra Leone Ministry of Health and Sanitation provided approval by the MOHS.

Informed Consent of participating subjects got first recorded at the community level during the syndromic survey. All adult members of households involved in the longitudinal study were asked to consent to participate and again asked about their willingness to donate a blood sample when they present with illness. Further consent was obtained during the sample collection for those subjects that visited the hospital when febrile.

3.4 RESULTS

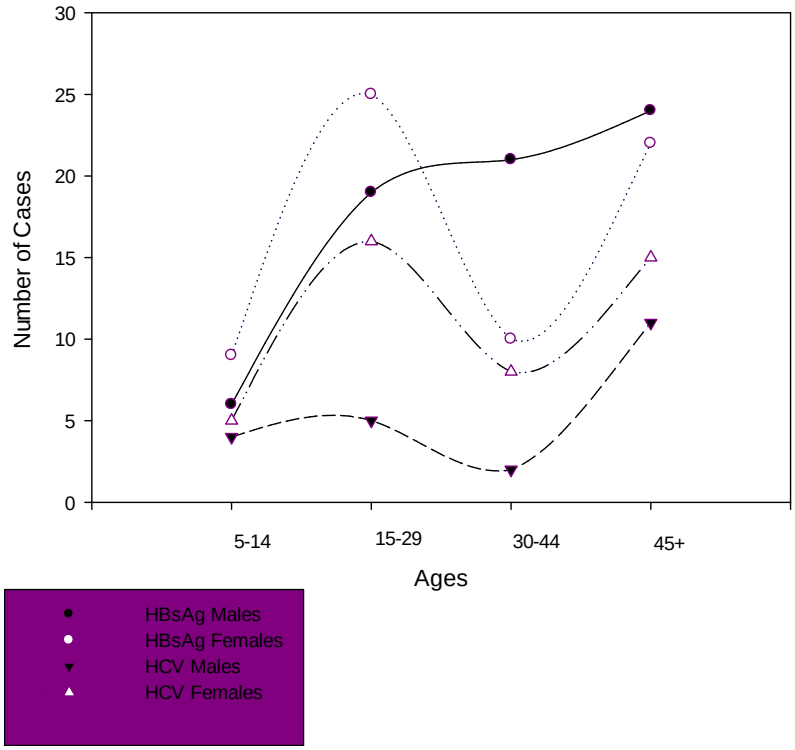
There was an average monthly attendance of 160.25 SD±10.63 for the twelve months of cohort study, with a significant difference in monthly attendances ($P<0.001$). The ages 5 to 14 years had an average of 25.83±7.85 attendances per month. Those within 15 to 29 years had an average monthly attendance of 48.83±6.7. Ages 30 to 44 had 41.66±4.2 while the age group ≥45 years had a monthly attendance of 43.91±6.7 (Table 3.1).

Table 3.1 Monthly attendance by febrile subjects

Month	5-14yrs	15-29yrs	30-44yrs	≥45yrs	Male	Female	Total
Jan	23	61	37	30	73	82	151
Feb	8	59	40	56	54	87	163
Mar	17	61	27	56	47	89	161
Apr	57	23	54	15	61	71	149
May	9	62	31	58	113	160	160
June	23	53	44	34	52	96	154
July	11	25	66	53	54	97	155
Aug	7	54	43	37	56	107	141
Sept	101	10	17	8	64	97	136
Oct	16	31	43	42	54	95	132
Nov	14	97	65	97	61	99	273
Dec	24	50	33	41	65	89	148
Mean± SD	25.8±27.2	48.8±23.4	41.7±14.6	43.9±23.1	62.8±17.3	97.4±21.8	160.3±36.8
CI of Mean	±17.3	±14.8	±9.3	±14.7	±11	±13.8	±23.4
Midian	16.5	53.5	41.5	41.5	58.5	95.5	152.5
Total febrile episodes	310	586	500	527	754	1169	1923

There was a monthly average of 10.4±1.9SD for Hepatitis B; 5.5±2.5SD for Hepatitis C (Fig 4.1) and 8.9(±3.4SD) for Anti-Hepatitis A. The prevalence of hepatitis B was 9.7% and hepatitis C, 4.7% while seroprevalence of Hepatitis A was 8.7%. Two age groups, 15-29 years, and ≥ 45years had higher rates of both HCV and HBV while more females were infected than males($P<0.05$) (Fig.3.2).

Fig 3.2: Age and Sex Distribution of Viral Hepatitis in Bo, Sierra Leone



Syndromically, 32% fever, 22% cough, 15% headache and 16% abdominal pain were the commonest syndromes of viral hepatitis among male subjects (Fig 3.3A). In female subjects, fever (32%), headache (18%), abdominal pain (18%) cough (15%) were the commonest syndromes (Fig3.3B). However, these syndromes are not unique to viral hepatitis and may not be precise.

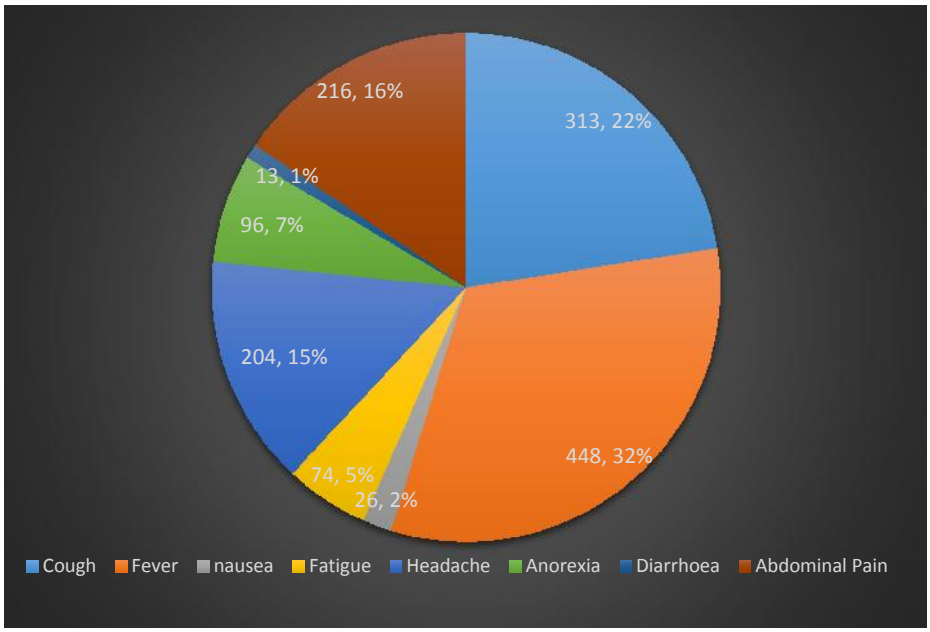


Fig 3.3A Syndromes of Viral Hepatitis in Males in Bo, Sierra Leone

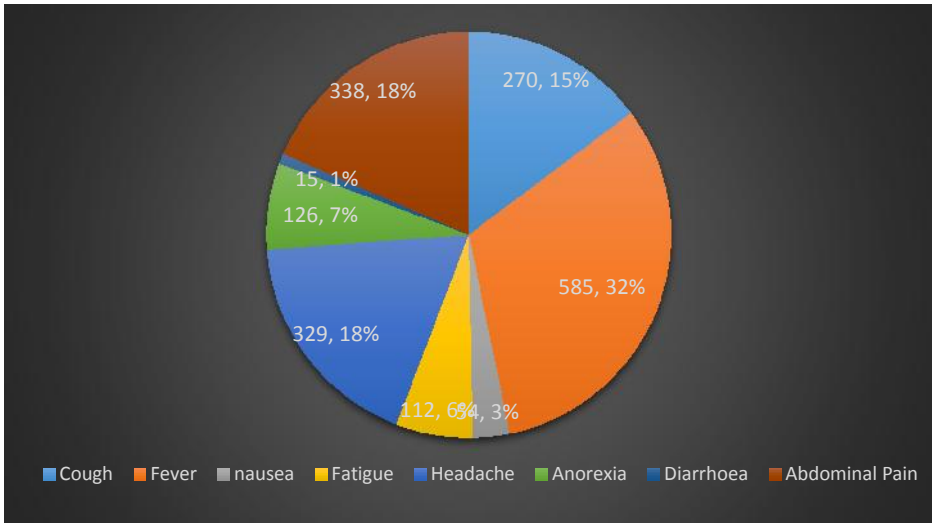


Fig 3.3B Syndromes of Viral Hepatitis in Females in Bo, Sierra Leone

Furthermore, in the follow-up seroprevalence study that was done in 2014, 175 febrile subjects were recruited at Mercy Hospital. They had an average age of 31.4 years, and 80(44.7%) of them, females, were tested for hepatitis A, B, and C. About 51.43% (95%CI:

44.07-58.72%) were positive for hepatitis B envelope antibody (HbEab), 49.71 % (95%CI: 42.39 -57.04%) were positive for HBV core antibody (HbCAb) and only 1.71% (95%CI: 0.58-4.91%) and 1.14 %(95%CI: 0.31-4.1%) were positive for HBV surface antigen (HBsAg) and HBV surface antibody (HBsAB) respectively. Further, 1.14 %(95%CI: 0.31-4.1%) were positive for hepatitis C.

In addition, urinalyses were done on 1,215 urine samples obtained from subjects during the cohort study. The average specific gravity was 1.94±0.85SE. The normal range for specific gravity of urine is 1.003-1.020[17], this high average range is indicative of high urine concentration or dehydration of subjects. About 50.04% (95% CI 47.21% -52.85%) were cloudy; 10.21 %(95%CI 8.63-12.04%) had proteinuria and 57.7 %(95%CI: 54.9%-60.45%) had traces of protein in their urine. Though dark or cloudy urine could mean infections of other etiologies, viral hepatitis has been associated with dark urine[18] and protienuria[19].

3.5 DISCUSSION

The results demonstrate a high incidence of viral hepatitis in the cohort population in Bo Sierra Leone. About 100 out of every 1000 persons are affected per year while about 50 out of every 1000 persons per year are affected by Hepatitis C.

The results are consistent with existing data for viral hepatitis, where HBV[12,19] infects up to 8-15% and about 2-3% are affected globally by HCV[13] and HAV is reported to be in transition in West Africa[20].

However, HCV, believed to have originated from West Africa,[21] remains a cause of morbidity in Bo, Sierra Leone. The seroprevalence of HCV in Bo is higher than the global seroprevalence which has increased in the past 15 years to 2.8% (95% UI: 2.6%-3.1%)[22] but consistent with the West African rates of 4.7 to 8.8%[22]. There are currently lot of treatment options for HCV in developed countries[23–26], but poor countries such as Sierra Leone lack adequate therapeutics for HCV and the limited supplies are provided at exorbitant rates which are unaffordable by many of the countries residents which live under 2USD a day. Having co-infections may affect treatment outcome adversely thereby making HCV positive cases at high risk of secondary diseases.

In the case of HBV, several studies in Sierra Leone have reported discordant prevalence on the seroprevalence of HBsAg. In 2005, Wurie[10] reported a seroprevalence of 6.3%(19/302)

of HBsAg among Sierra Leonean pregnant women of middle and high socioeconomic status and 11.2%(20/179) HBsAg in peri-urban and rural Sierra Leonean pregnant women indicating a higher prevalence in poorer settlements within the same country. In 2010, Solayide[27] reported a seroprevalence of 21.7%(43/198) of HBsAg in Freetown. In 2014, Koroma and Kangbais reported a seroprevalence of 47.5 %(1054/2218) in Bo Sierra Leone. Our result of 9.7% seroprevalence of Hepatitis B is consistent with the findings of Wurie[10] but contrary to the results of Koroma and Kangbai[8], who reported a 47.5% seroprevalence of HBsAg among women in Bo Sierra Leone from samples tested from May 2012 to June 2013 at the Bo Government Hospital. The study was done almost in parallel with this study and from within the same township. However, their study was based on clinical records and it is hard to tell if the records were entered correctly and included all persons that were tested for HBsAg.

However, in a follow-up study that was done in 2014, 51% (90/175) of persons screened had a history of Hepatitis B infection while only 1.41% were positive for Hepatitis C and only 1.41% tested positive for HBsAg. While new infections were low, the additionally data demonstrated a high seroprevalence of viral hepatis B consistent with the data by Koroma and Kangbai[8].

The disease incidence during the one year cohort study seems to have been higher generally in Bo, Sierra Leone especially in the cohort study communities which are generally poor, having houses occupied by multiple families with an average of 6 per household and 18 per house.[28]

3.6 CONCLUSION

For every one person affected by hepatitis C in Bo, Sierra Leone, two individuals are affected by Hepatitis B, with no risk of infection from Hepatitis A. This incidence recorded in the period of July 2012 to June 2013, may differ from incidences recorded at other times in the future. The lack of new cases of hepatitis A within the cohort demonstrates a better environmental health within the catchment population of Mercy Hospital where this study was carried out, as poor sanitation generally contributes to HAV transmission.

Notwithstanding, viral hepatitis remains a major contributor to illnesses and death in resource-poor countries such as Sierra Leone, and the incidence of the disease seems to

fluctuate with time and space. The treatment options in Sierra Leone are fewer and hard-to-get for infected persons.

3.7 REFERENCES

1. Gallagher KM, Novak RT. Hepatitis, viral. In: International Encyclopedia of Public Health. 2008. p. 374–82.
2. Moradpour D, Blum HE. [Viral hepatitis]. Ther Umsch [Internet] 2011;68:175–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21452137
3. Lai CL, Ratziu V, Yuen M-F, Poynard T. Viral hepatitis B. Lancet 2003;362:2089–94.
4. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. Lancet 2003;362:2095–100.
5. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. Vaccine 2010;28:6653–7.
6. Lemoine M, Thursz M, Njie R, Dusheiko G. Forgotten, not neglected: Viral hepatitis in resource-limited settings, recall for action. Liver Int 2014;34(1):12–5.
7. Abbas Z, Siddiqui AR. Management of hepatitis B in developing countries. World J Hepatol [Internet] 2011;3:292–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3246547&tool=pmcentrez&rendertype=abstract
8. Mohamed Koroma and Jia B Kangbai. Seroprevalence of HBsAg among Female Patients Seeking Healthcare in Bo Government Hospital, Southern Sierra Leone: 14-Months Database Study. Int J Trop Dis Heal [Internet] 2014;4(6):713–22. Available from: www.sciencedomain.org
9. Torlesse H, Wurie IM, Hodges M. The use of immunochromatography test cards in the diagnosis of hepatitis B surface antigen among pregnant women in West Africa. Br J Biomed Sci 1997;54:256–9.
10. Wurie IM, Wurie AT, Gevao SM. Sero-prevalence of hepatitis B virus among middle to high socio-economic antenatal population in Sierra Leone. West Afr J Med [Internet] 2005 [cited 2014 May 17];24(1):18–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15909704

11. Ganem D. Virology. The X files--one step closer to closure. Science 2001;294:2299–300.

12. Kwon SY, Lee CH. Epidemiology and prevention of hepatitis B virus infection. Korean J Hepatol 2011;17:87–95.

13. Lemoine M, Nayagam S, Thursz M. Viral hepatitis in resource-limited countries and access to antiviral therapies: current and future challenges. Future Virol 2013;8:371–80.

14. B.C. L, G.G. F, A. von B, Langer BC, Frosner GG, von Brunn A. Epidemiological study of viral hepatitis types A, B, C, D and E among Inuits in West Greenland. J Viral Hepat [Internet] 1997;4:339–49. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med4&NEWS=N&AN=9310933\nhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed4&NEWS=N&AN=9310933

15. Herrera S, Vallejo AF, Quintero JP, Arévalo-Herrera M, Cancino M, Ferro S. Field evaluation of an automated RDT reader and data management device for Plasmodium falciparum/Plasmodium vivax malaria in endemic areas of Colombia. Malar J [Internet] 2014 [cited 2014 Sep 25];13:87. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3995821&tool=pmcentrez&rendertype=abstract

16. Shekalaghe S, Cancino M, Mavere C, et al. Clinical performance of an automated reader in interpreting malaria rapid diagnostic tests in Tanzania. Malar J [Internet] 2013;12:141. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23617722\nhttp://www.biomedcentral.com/content/pdf/1475-2875-12-141.pdf

17. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. Am Fam Physician [Internet] 2005 [cited 2014 Sep 24];71(6):1153–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15791892

18. Prakash C, Thomas J, Kumari S, Verghese T. Non Hepatitis Viruses in Causation of Acute Sporadic Non-A, Non-B Viral Hepatitis. J Commun Dis 1996;28:185–8.

19. Huang JF, Chuang WL, Dai CY, et al. Viral hepatitis and proteinuria in an area endemic for hepatitis B and C infections: Another chain of link? J Intern Med 2006;260:255–62.

20. Jacobsen KH. Hepatitis A virus in West Africa: Is an epidemiological transition beginning? Niger Med J [Internet] 2014 [cited 2014 Sep 24];55(4):279–84. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4124538&tool=pmcentrez&rendertype=abstract

21. Magiorkinis G, Magiorkinis E, Paraskevis D, et al. The global spread of hepatitis C virus 1a and 1b: A phylodynamic and phylogeographic analysis. PLoS Med 2009;6(12).

22. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013;57(4):1333–42.

23. Liang TJ, Ghany MG. Current and Future Therapies for Hepatitis C Virus Infection [Internet]. N Engl J Med. 2013;1907–17. Available from: http://www.nejm.org/doi/full/10.1056/NEJMra1213651

24. Anantharamu T, Brashier D, Dahiya N, Gupta A, Sharma S, Sharma A. A review of the current anti-HCV therapy: Are we finally ready for interferon-free regimens. Int J Nutr Pharmacol Neurol Dis [Internet] 2014;4(5):6. Available from: http://www.ijnpnd.com/text.asp?2014/4/5/6/147455

25. Hatzakis A, Chulanov V, Gadano AC, et al. The present and future disease burden of hepatitis C virus (HCV) infections with today ' s treatment paradigm – volume 2. 2015;

26. Charlebois A, Lee L, Cooper E, Mason K, Powis J. Factors associated with HCV antiviral treatment uptake among participants of a community-based HCV programme for marginalized patients. J Viral Hepat 2012;19(12):836–42.

27. Solayide A Adesida, Gevao F Tamba, Foday Sahr and GMS. Seroprevalence of Human Immunodeficiency and Hepatitis B Viruses among Patients at a Health Facility in Freetown, Sierra Leone. Sierra Leone J Biomed Res 2010;2(1):28–31.

28. Ansumana R, Malanoski AP, Bockarie AS, et al. Enabling methods for community health mapping in developing countries. Int J Health Geogr 2010;9:56.

COMMON FEBRILE INFECTIONS IN SIERRA LEONE: RESPIRATORY INFECTIONS.

4.1 ABSTRACT

Background: Respiratory infections constitute an important non-malaria febrile condition[4], contributing to over 17% of deaths of children under five[6] and has a significant proportion of the 85% of deaths caused by communicable diseases in Sierra Leone

The aim of this study was to investigate respiratory pathogens using a tiered laboratory analyzes approach in Bo Sierra Leone. Syndromes of respiratory infections within the catchment of Mercy Hospital were assessed.

Methods: This study was part of a cohort study that lasted for 12 months starting in July 2012 up to June 2013. A syndromic survey preceded the respiratory sample collection within the catchment population of Mercy Hospital in Bo Sierra Leone. In addition, syndromic surveillance was done using data synchronized by the monitoring system.Throat swabs and nasal swabs were collected from cohort subjects and tested using three tiers of analyzes. Tier 1 involved the use of lateral flow immunoassays, tier2: multiplex PCR and tier 3: resequencing pathogen microarray.

Results:

Syndromically, there were 38.62 % (821/2126**;** with 95% CI: 36.57-40.71%) respiratory syndromes in Bo for the twelve month period between July 2012 and June 2013. Based on syndromes, children between 6 and 14 years were 44.44% (104.00/234) prevalent (95%CI: 38.21-50.85); 32.6% (CI 28.64-36.83) for 15-29 year olds; 39.55 %(CI, 32.06-42.54) for those between 30 to 44 years; and 37.15 %(CI, 32.06-42.54) for those that were either 45 years or above.

There were 10(8.3%) detections of Influenza B and 2(1.7%) of pandemic flu H1N1, 29 cases of Human Rhino virus/enterovirus(24.2%), 23 cases of Corona Virus (19.2%), 7 cases of bacterial *Chlamydophila pneumonia*(5.8%), 6 cases of adenovirus(5%), 2 Parainfluenza 1(1.7%),3 bacterial *Mycoplasma pneumonia*(2.5%). Other detections were *Streptococcus*

pneumonia, Haemophilus influenza, Moraxella catharrhalis, Klebsiella pneumonia, and Cytomegalovirus.

Conclusion

The high syndromic prevalence of respiratory infections suggests that these type of infections constitute a standard non-malarial condition in Bo Sierra Leone. Further, both viruses and bacteria remain important etiologic agents of respiratory infections. Further studies are required to compare the diagnostic capabilities of the multiplex PCR system and RPM, where discrepant results were observed.

4.2 INTRODUCTION

Apart from malaria, several common febrile illnesses in Sierra Leone contribute to disease morbidity and mortality [1,2,3,4,5]. Respiratory infections constitute an important non-malaria febrile condition[4], contributing to over 17% of deaths of children under five[6] and has a significant proportion of the 85% of deaths caused by communicable diseases in Sierra Leone [7]. All infections of the respiratory tract are respiratory infections [8,9] with the upper respiratory tract (URT) comprising the nose, paranasal sinuses, middle ear, nasopharynx, oropharynx and laryngopharynx [10]. Infections of the upper respiratory pathways include the common cold, pharyngitis, tonsillitis, pharyngotonsilitis, sinusitis, rhinosinusitis, epiglottitis, and laryngotracheitis; which are usually benign, transitory and self-limited or intense diseases [9,11].

The lower respiratory tract (LRT) on the other hand, is all the part of the respiratory pathway that is below the vocal cords and includes the tracheae, bronchi, bronchioles and alveoli air sacs. Infections of the lower respiratory tract are called lower respiratory infections, including; laryngotracheobronchitis with a syndrome called croup, bronchiolitis, pneumonia and influenza infections, [10,12–16]. Respiratory infections could also be classified based on their symptomatology into acute respiratory or mild respiratory infections, [8,9].

However, there is scarcity of data on respiratory infections in Sierra Leone[17]. Notwithstanding, the global report on respiratory infections is grim and acute respiratory infections (ARI) are eminent contributors to illnesses and death. ARIs have a worldwide disease burden estimated at 112,900,000 disability adjusted life years and 3.5 million deaths, mostly in resource-poor countries such as Sierra Leone [18, 19]. Of the deaths caused by ARIs, about 2 million are children under five years corresponding to about 19% of all deaths in this age group[20,21].

The causative agents of upper and lower respiratory tract infections encompass several pathogens. The upper respiratory tract has normal flora consisting of *Streptococcus pneumonia*, anaerobic and *microaerophilic streptococci*, *Streptococcus milleri* (found in sinuses), *Haemophilus influenzae* and other *Haemophilus sp.* Diphtheroids, coagulase-

negative staphylococci, *S. aureus*, *Moraxella catarrhalis* and *Neisseria spp. Prevottela melaninogenicus* and other species [10].

Notwithstanding the cause of the illness, definitive diagnoses in resource poor settings for respiratory infections is barely available. Clinicians use their experience and knowledge of predictors or syndromes of respiratory infections to treat infected persons. It may not suffice in an advanced era where pathogens change form, and there are molecular platforms to detect the pathogens involved. Detecting a pathogen as part of the etiologic agents in the respiratory disease ecology of a resource-poor location, necessitates simpler and cheaper kits for testing respiratory pathogens in austere environments.

4.3 GOAL

The aim of this study was to investigate respiratory pathogens using a tiered laboratory analyzes approach in Bo Sierra Leone. Syndromes of respiratory infections within the catchment of Mercy Hospital were assessed.

4.4 METHODS

This study was part of a cohort study that lasted for 12 months starting in July 2012 up to June 2013. A syndromic survey preceded the respiratory sample collection within the catchment population of Mercy Hospital in Bo Sierra Leone. In addition, syndromic surveillance was done using data synchronized by the monitoring system.

Throat swabs and nasal swabs were collected from cohort subjects and tested using three tiers of analyzes.

Fig. 4.1: Respiratory Sample Collection

First tier comprised of lateral flow immunoassays (LFIs) or immunochromatographic tests (ICT). Second tier was by the FilmArray multiplex polymerase chain reaction (PCR), and the final tier was resequencing pathogen microarray using RPM Flu Chip 3.1.

4.4.1 Tier 1: Lateral Flow Immunoassays Method

The LFIs used were the multiplex Influenza A, Influenza B and Pandemic Flu (H1N1) tests; Streptococcus aureus LFI and respiratory syncytial virus (RSV). Swabs collected were in duplicate: two throat swabs and two nasal swabs per subject. One of the two sets of collected swab samples were dipped and swirled each in a 1.5ml Eppendorf tube containing 400ul of saline solution for molecular analyzes and the other used for rapid lateral flow immunoassays.

SD BIOLINE Influenza Ag A/ B/ A (H1N1) Pandemic was used. The nasal swabs obtained from subjects and immersed in the sample buffer provided with the kit as directed by package insert. The test strip was then removed and inserted into the tube to sample and buffer and result acquired at 15 minutes. Positive and negative controls included in the assays were also done to assess the quality of the assay.

For *Streptococcus aureus*, only throat swabs were eligible for sample types as described in the package insert of the SD Bioline test kit. The kit detects viable or nonviable organisms directly from the throat swabs within 5-10mins with a sensitivity and specificity of 87.3% and 95.8% respectively. After placing the throat swab in a test tube provided with the kit, three drops of Extraction Reagent A was added. This was followed by three drops of Extraction Reagent B and swirled before inserting test strip and waiting for 5-10 minutes for the result.

The SD Bioline RSV test kit was used for detecting antigens of respiratory syncytial virus. The Kit has a sensitivity of 92.3% and a Specificity of 93.3%. To conduct a test, about 200-250 µl of assay diluent was added to the provided test tube as directed by insert. The swab was dipped into diluent and swirled, after 10 minutes of waiting for it to settle down, test strip was added and result obtained within 15 minutes.

4.2.2 TIER 2: MULTIPLEX PCR RESPIRATORY PANEL

The FilmArray system (Biofire Diagnostics, Salk Lake City,Utah, USA), is a multiplexed PCR in vitro diagnostic system that is also a lab-in-a-pouch system (Fig 4.2) that performs a

mesoscale (0.001 to 0.2ml working volume) sample fluid manipulation in a self-contained, disposable thin-film plastic pouch[22].

Fig 4.2: The FilmArray System

The FilmArray Respiratory Panel (FRP) assay carries out an automated, rapid, nested multiplex PCR using two target controls; one RNA target from *Schizosaccharomyces pombe* and a DNA target, which is included in the wells and controls for the second-stage PCR[22]. It simultaneously detects any of 20 respiratory pathogens including 17 viruses and their subtypes and three bacteria pathogens in a single respiratory specimen. The device can detect pathogens based on both complete genome sequences and partial gene sequences. The list of pathogens detected by the FDA approved respiratory panel include:

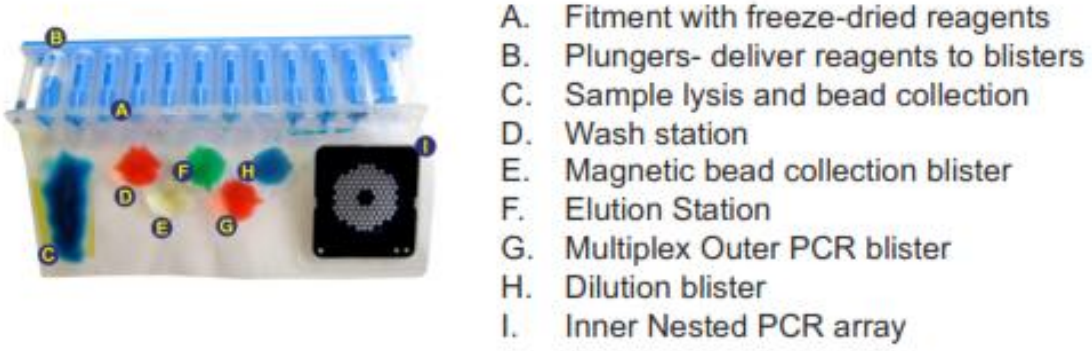
Table 4.1: list of pathogens detected by the FDA approved respiratory panel

Influenza A	*FluA/H1 subtyping*	*Coronavirus NL63*
Influenza B	*FluA/H3 subtyping*	*Coronavirus HKU1*
Parainfluenza virus 1	*FluA/H1-2009 subtyping*	*Coronavirus OC43*
Parainfluenza virus 2	*Human Rhinovirus/ Enterovirus*	*Human Metapneumovirus*
Parainfluenza virus 3	*BocavirusS*	Adenovirus
Parainfluenza virus 4	*Coronavirus 229E*	*Bordetella pertussis*
Respiratory Syncytial Virus		Chlamydophila pneumonia

The FilmArray does nested multiplex PCR without amplicon contamination. The pouch is a closed system and up to 120 second-stage PCRs can be performed in the pouch, enough for multiple tests for several pathogens and still include controls for process validation. Conserved sequences from specific gene segments of proteins or 5' UTR regions were selected as assay targets in the assay. Subsequent to injection of the sample into the pouch, are: (1) sample lysis (2) nucleic acid purification (3) cDNA synthesis and outer multiplex PCR (4) automated nested PCR and (5) amplicon melt analysis[23].

The FRP pouch (Fig 4.3) and its physical design has been described extensively by Babady et al. [22] It contains all of the freeze-dried reagents (primers, buffers, enzymes, LCGreen Plus dye, etc.) required for nucleic acid extraction, nested RT-PCR, and high resolution melting[24] and is the site of biological processes that allow the device to manipulate quantification of sample nucleic acids for screening of pathogen markers. Three pneumatic elements within the instrument, including the pistons and the plungers in the top of the fitment control the movement of liquid throughout in the pouch.

Figure 4.3: An Annotated FilmArray Pouch



All of the biochemical reagents used for reservoirs and PCR arrays are freeze-dried into 12 wells of the fitment. From left to right:

Well 1: PCR Process control material (*Schizosaccharomyces pombe* cells)

Wells 2, 3, 4, 5: Sample Wash Buffer

Well 6: Nucleic acid elution buffer

Well 7: Reverse transcription/first stage PCR master mix

Well 8: Dilution buffer

Wells 9, 10: Second stage PCR master mix[22]

Well 11: Empty

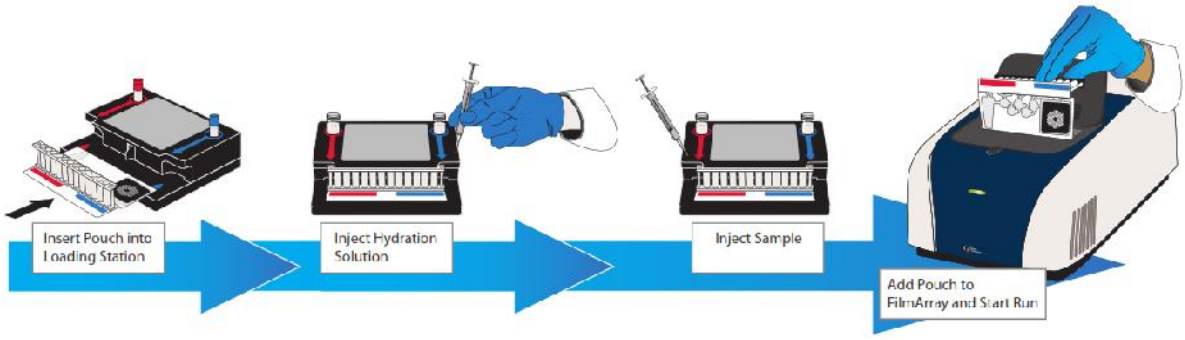
Well 12: Overflow reservoir for the second stage PCR mix[22]

In the lysis chamber, through a process called bead beating, the FilmArray breaks apart any cells or viruses in the sample. Ceramic beads, agitated in high speeds, break open cells and viruses and release the nucleic acids. These acids bound to magnetic beads, which then proceed into the purification chamber. A wash buffer eliminates any cellular or viral debris while a magnetic field keeps the beads in place. In this stage, an elution buffer releases the bound nucleic acids. A reverse transcription is then initiated to convert any target RNA fragments into DNA. Afterward, a first-stage PCR with specific primer pairs are used to amplify fragments, and the remaining products are then further amplified if possible during the second-stage PCR step. The final products are aliquot and tagged with fluorescent antibodies for detection (BioFire). As shown below, analysis of these products can give identification for the following pathogens.

4.2.2.3 FilmArray Sample Processing Procedures

The Food and Drug Administration approved sample for the FilmArray, nasal swabs, were used, and samples extracted by swirling tips of swabs into 0.1M saline solution. The FilmArray test kit is provided with two vials, a red sample vial, and a blue hydration buffer vial. The hydration solution in blue vial was first added to the pouch, using the buffer syringe (blue) to rehydrate the freeze-dried reagents. Unprocessed sample was mixed directly with the sample buffer in red vial. The sample-buffer mix was injected into the pouch using the sample buffer syringe. The prepared pouch was inserted into the FilmArray instrument, barcode of pouch registered by the barcode reader and a run initiated. Results are available in approximately 1 hour.

Fig 4.4: FilmArray Sample Processing Steps



The instrument performs individual second-stage PCR reactions and perform high-resolution melting analysis. The FA software algorithms automatically determines if the analytes are detected in the specimen,[24] based on the melting profile of the PCR products.

4.2.3 Resequencing pathogen microarray (RPM-FLU V.3.1)

The resequencing pathogen microarray (RPM) system (Fig 4.5) was used in this study. Flu chip RPM-Flu v.3.1 was utilized in this study. A Resequencing Pathogen Microarray (RPM) is highly multiplexed assay for detecting and differentiating similarly related etiologic agents by using carefully overlapping probe sets to determine a target organism's nucleotide[25,26]. RPM has different chips with different targets on them, but the flu chip (Fig 4.6) can detect over 30 viral and bacterial pathogens. Because it is a resequencing array, it can also detect emerging pathogens or near neighbors of existing pathogens.

Fig. 4.5: Rpm System in Bo Sierra Leone

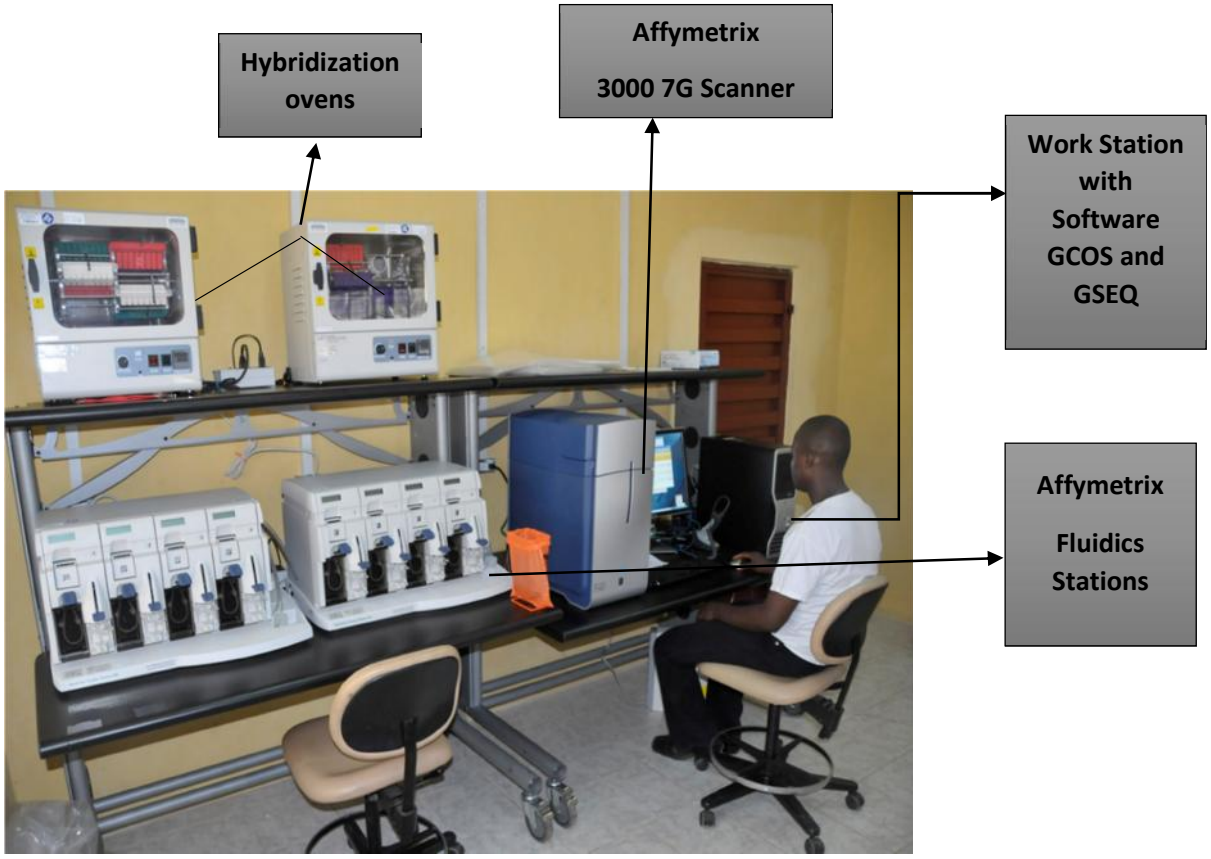
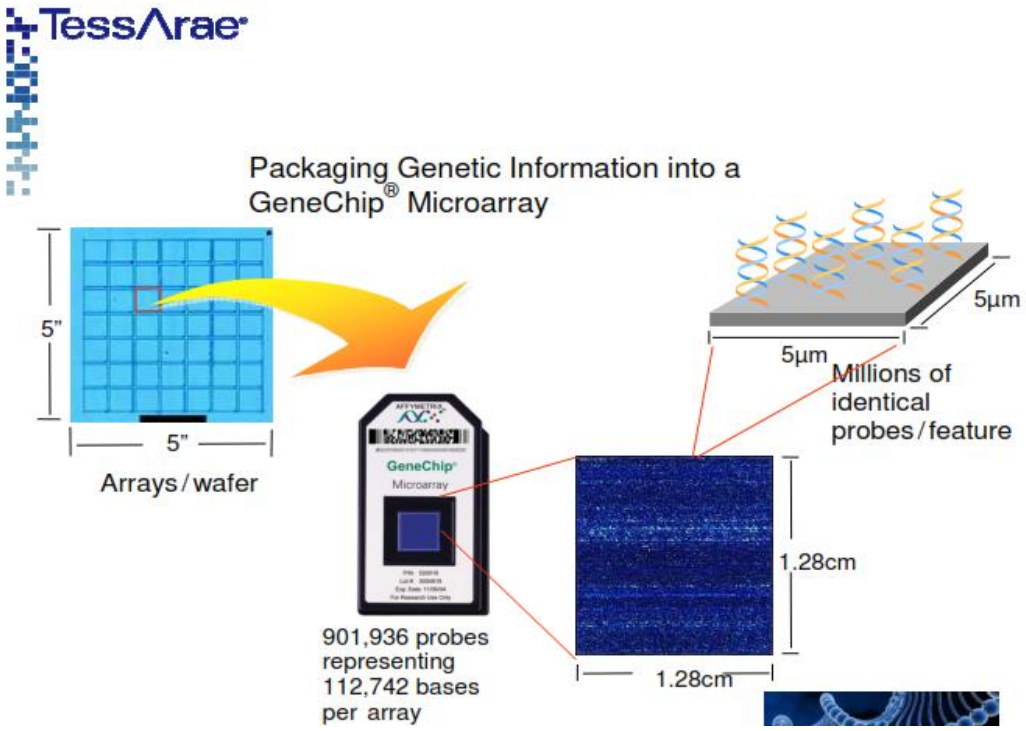
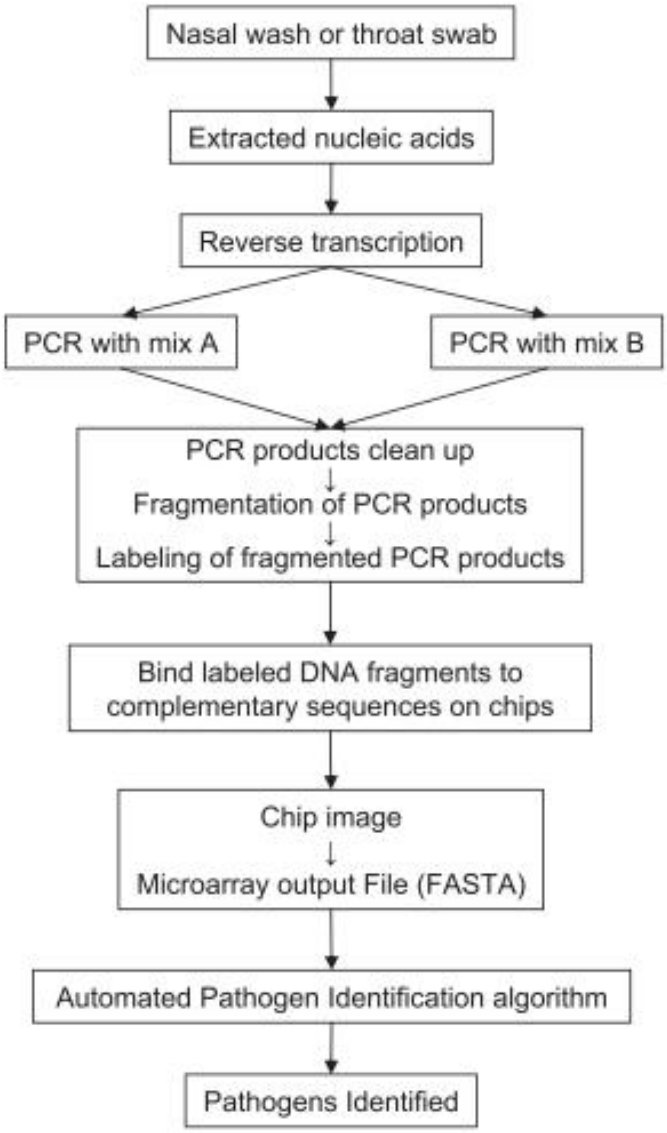


Fig 4.6: RPM Chip Design



Source: Tessarae.LLC

Fig 4.7: RPM Procedures (Lin et al[26])



RPM technology operates a multistep process: (1) nucleic acid extraction of either DNA, RNA, or both (2) sample amplification via whole genome amplification or multiplex PCR (3) labeling (often preceded by fragmentation) (4) microarray hybridization (5) microarray scanning.

4.2.3.1 Specimen collection and processing

Sterile swabs were used to obtain a sample from throat and nostrils. The swabs were dipped in 400ul of saline solution and 150ul of the solution spiked on Whatman protein-saver Fast Technology for Analysis (FTA) cards. The FTA cards protect nucleic acid samples from degradation by nucleases and other processes[27]. They are integrated with a weak base, chelating agent, anionic surfactant or detergent, and uric acid (or a urate salt) imbedded into a cellulose-based matrix. Moreover, they are proven for adequately storing DNA and RNA samples at room temperature for weeks or years without noticeable degradation[27].

As positive controls, live influenza strains H1N1 and H3N2 virus samples at different concentrations (0.1 pg/µl and 0.01 pg/µl for each) were stored on FTA cards. FTA cards inactivate all pathogens and do not need to be stored under BSL-2 conditions[27].

4.2.3.2 Primer Design

Four independent multiplex primer mixes, separated to simplify primer design and enhance multiplex optimization, were used to amplify targeted sequences on RPM-Flu v.3.1 microarrays. To accommodate the genetic variability of RNA-based and DNA-based viruses and ensure amplification of even closely related strains, a software-script automated a primer selection algorithm to select primers by unique primer regions. Each of the four multiplex primer cocktails dedicated to the detection and amplification of a class of pathogens: influenza, other viruses, bacteria, and entero/rhinovirus species.

4.2.3.3 Multiplex RT-PCR amplification

Following 2µl of cDNA reverse transcription (25ºC, 10 min; 50ºC, 50 min; 85ºC, 5 min), the obtained cDNA was apportioned into four independent aliquots of equal volume for each sample type. Four separate multiplex PCR reactions, as described above, were conducted with specific primer cocktails to amplify all detectable targets. For this step, from the master mix of each cocktail solution, 45µl were added to 4.9µl of the cDNA. Each prepared sample solution was incubated in the thermal cycling protocol (24ºC, 10 min; 94ºC, 2 min; 16 cycles

[94ºC, 30 sec; 45ºC, 30 sec; 72ºC, 90 sec]; 24 cycles [94ºC, 30 sec; 96ºC, 30 sec; 60ºC, 2 min]).

4.2.3.4 Microarray Hybridization and Analysis

PCR amplification products were recombined and purified using Qiagen purification columns. Purified products were fragmented using DNase I and labeled with biotinylated ddATP via terminal transferase. These mixes were incubated (37ºC, 30 min; 95ºC, 5 min). RPM-Flu v.3.1 microarray chips were pre-heated in the oven with pre-hybridization buffer solution for 12 minutes at 60 rpm. 96µl of hybridization solution added to 34.5 of the fragmented and labeled PCR products. Once loaded, the microarray chips were incubated at 95ºC for 5 minutes and then 49ºC for another 5 minutes. Final hybridization of chips ran overnight at 49ºC, 60 rpm.

On the molecular level, overlapping 25-nucleotide probe sets inside the chips shifted by one base nucleotide are generated for the whole prototype sequence. Each probe set contains one matching and three mismatched probes differing only by the middle nucleotide for each resequenced base of the prototype sequence. The probes for each probe set occupy four neighboring spots on the microarray. The differential hybridization to probes in each configured to labeled fragments of the target allows the sequence determination. The base calls for detected target fragments are made by resequencing adapted software base on the relative strength of hybridization to probes within each set. In this way, the resequencing microarray detects related targets containing single nucleotide polymorphisms.

After hybridization, the samples were then removed from the microarray cartridges, and hybridization buffer was reloaded into each chip. The chips were washed and stained using SAPE stain solution, antibody mixes, and array washing buffer at a GeneChip 450 microfluidics station. A GeneChip 3000 scanner was used to scan and analyze the microarrays. Scan results, which included sequencing data based on microarray hybridization patterns via fluorescence and *in situ* probe detection, were generated with GeneChip software. Sequences, including notations of mismatched gene signatures and single nucleotide polymorphisms indicative of mutations, were used to produce FASTA

output files. FASTA files were submitted for analysis to Tessarae using a proprietary identification algorithm and BLAST search of the Genebank database.

RESULTS

4.3.1 Syndromic Distribution of Respiratory Infections

Syndromically, there were 38.62 % (821/2126**;** with 95% CI: 36.57-40.71%) respiratory syndromes in Bo for the twelve month period between July 2012 and June 2013. There were no significant differences by sex (P>0.05). Age wise, there were 41.22 % (277/672) prevalence (95% CI, 37.56-44.98) for children under five years of age. Children between 6 and 14 years were 44.44% (104.00/234) prevalent (95%CI: 38.21-50.85); 32.6% (CI 28.64-36.83) for 15-29 year olds; 39.55 %(CI, 32.06-42.54) for those between 30 to 44 years; and 37.15 %(CI, 32.06-42.54) for those that were either 45 years or above (Table 4.2). (Note that this result includes 672 children that were not part of the cohort of 1403 and 51 recurrences of respiratory syndromes)

Table 4.2: Distribution of Respiratory Syndromes from the Catchment of Mercy Hospital in Bo, Sierra Leone.

Age	**Overall**				**Males**				**Females**				P-Value of Chi Squared tests for differences by sex
	N	**Respiratory Syndromes (RS)**	**% of Syndromes (%RS)**	95%CI	**N**	**RS**	**% RS**	95%CI	**N**	**RS**	**%RS**	95%CI	
0-5	672	277	41.2	37.6-45.0	262	109	41.6	35.8-47.7	410	168	41.0	36.3-45.8	0.9
6-14	234	104	44.4	38.2-50.9	109	48	44 0	35 1-53 4	125	56.0	44.8	36.4-53.5	09
15-29	500	163	32.6	28.6-36.8	180	63	35.0	28.4-42.2	320	100	31.3	26.4-36.5	0.4
30-44	397	157	39.6	34.9-44.4	186	74	39.8	33.0-47.0	211	83.0	39.3	33.0-46.1	0.9
≥45	323	120	37.2	32.1-42.5	164	65	39.6	32.5-47.3	1S9	55.0	34.6	27.6-42.3	0.3
Mean	425.2	164.2	38.9	34.1-43.3	180.2	71.8	40.0	33.0-47.1	245	92.4	38.2	31.9-43.9	
±SD	169.04	67.74	4.0		54.9	22.8	3.3		118.1	46.3	5.3		
Total	2126	821	38.6	36.6-40.7	901	359	39.8	36.7-43.1	1225	462	37.7	35.0-40.5	P>0.05

4.3.2 Tiered Analyzes of Respiratory Samples

Of the respiratory syndromes encountered, 120 were tested. All were tested by rapid lateral flow immunoassays and rapid automated PCR. 16 of the 120 samples, about 13% were selected, and this set was tested using RPM-Flu v.3.1. From the RLFI, only the multiplex influenza RFLI had detections. There were 10(8.3%) detections of Influenza B and 2(1.7%) detections of pandemic flu H1N1 in Bo Sierra Leone. The other two, *Streptococcus aureus* and RSV kits had no detections, and the higher tier tests did not also pick up RSV or Strep A.

At Tier 2, the following pathogens were detected: 29 cases of Human Rhino virus/enterovirus(24.2%), 23 cases of Corona Virus (19.2%), 7 cases of bacterial *Chlamydophila pneumonia*(5.8%), 6 cases of adenovirus(5%), 2 Parainfluenza 1(1.7%) and 3 bacterial *Mycoplasma pneumonia*(2.5%).

At Tier 3 six pathogens were detected using RPM-Flu v.3.1. The detections were *Streptococcus pneumonia* (76%) *Haemophilus influenza (53%) Moraxella catharrhalis (29%), Klebsiella pneumonia, Cytomegalovirus and Influenza A (6% each)*

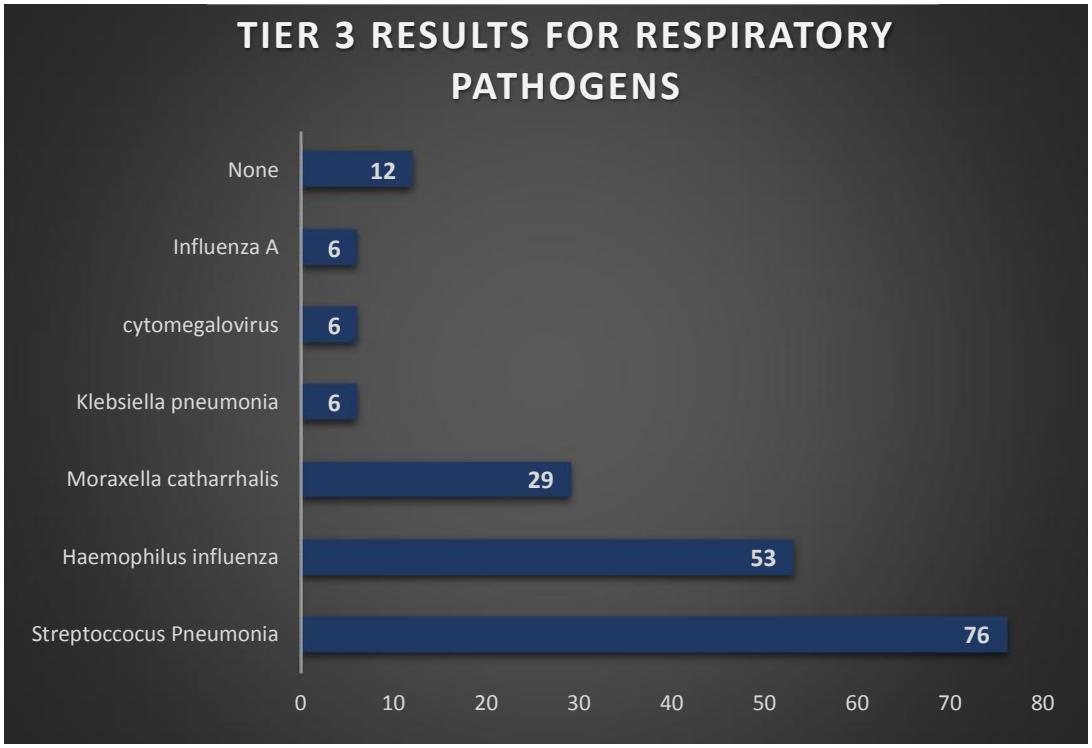


Fig 4.6. Data summary. 17 of 120 nasopharyngeal swab samples analyzed on RPM Flu v.3.1 to detect and identify possible infectious respiratory pathogens.

There was a 100% concordance between RSV rapid lateral flow immunoassay (SD Bioline, Seoul, South Korea) and the FilmArray PCR diagnostic platform for 120 tests and the RPM platform for 17 tests that were compared. The RSV kit tested negative for 120 samples screened. The PCR also tested negative for the same 120 samples screened for RSV. In the case of RPM, only 17 tests were accessed, and all were in agreement with the RSV kit. For all tests, negative controls tested negative and positive controls tested positive.

Similarly, there was a 100% concordance between the *Strep a* rapid lateral flow immunoassay (SD Bioline, Seoul, South Korea) and the FilmArray PCR diagnostic platform and the RPM platform. The Strep a kit tested negative for 120 samples screened. The PCR also tested negative for the same 120 samples screened for *Strep a*. In the case of RPM, only 17 tests were accessed, and all were in agreement with the Strep a kit.

However, there was no concordance between the multiplex influenza kit which tests for Influenza A (non-pandemic), Influenza A (H1N1), and Influenza B. The test did not agree with the PCR tests and the RPM tests.

Further, between the Tier 2 and Tier 3 assays, there were discordant results. While the RPM-Flu microarrays detected more bacterial pathogens, the multiplex PCR detected more viral pathogens. These bacterial *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis,* and *Klebsiella pneumonia were detections* on the RPM Flu chip with approximately 80% of the 17 samples tested by RPM testing positive for *S. pneumoniae*, whereas 40% of samples were positive for *H. influenzae*. Specifically, on analyzing sample TALRESP_001, while FilmArray detected Coronavirus OC43, the RPM-Flu v.3.1 detected *S. pneumoniae* and *H. influenzae*. In other instances, such as TLA_RESP_006, where FilmArray detected no pathogens present, RPM-Flu v.3.1 found targeted gene signatures for *S. pneumoniae*, *M. catarrhalis*, and *H. influenza*.

FilmArray is not designed to be able to detect the same broadband spectrum that RPM-Flu can detect. In fact, RPM detects and identifies even unknown strains of certain species based on resequencing advantages, but through a much more time-consuming process. Cases that are even further exacerbated by conditions like malaria can become even more severe or even fatal.

4.3.3. DISCUSSION

The high detections of viral pathogens in this study are not surprising though such a broad-spectrum etiology research has not been done previously in the study location. Most upper respiratory infections are of viral etiology[9]. In a study of viruses and bacteria in the etiology of the common cold, different strains of rhinoviruses were found to be the primary cause of the infection [28]. Further, it has been indicated previously that viruses such as coronavirus, influenza virus, respiratory syncytial virus (RSV), parainfluenza virus, adenovirus, and Metapneumovirus are contributing agents to common cold. [29]

It is important to understand detection of cytomegalovirus, which is one of the eight herpes viruses that affects humans, with a likelihood to infect immunocompromised patients[30]. The RPM also detected an Influenza A matrix gene that is generic and the strain of the Influenza A pathogen is unknown. Surprisingly, serotypes for influenza A can be detected using FilmArray. However, given that screening via FilmArray showed no presence of influenza serotypes, this affirms the broader detection scope and higher resolution of the RPM system. Influenza increases in clinical severity with increase in the age of the patient and persons with other health disorders, causing an intermittent seasonal morbidity and mortality worldwide[31]. The global burden of influenza varies every year with higher burdens in seasons when influenza A (H3N2) viruses predominate[32], rather than influenza A (H1N1) and B viruses[33,34].

It was surprising that there was no detection of RSV though investigated at the three tiers from the nasal and throat swabs. This poor performance of the tests for detecting RSV could be due to the sample type used; nasal and throat swabs instead of nasal wash or nasopharyngeal aspirate. Nasopharyngeal aspirate (NPA) or nasal wash specimens have been reported [35,36,37,] to be sufficient for sampling respiratory viruses though the use of NPAs is not ideal for children. In a comparative specimen sampling, using NPAs, throat swabs, (TS) and nasal swabs (NS) in the viral etiologies of acute respiratory infections; Do et al[38], reported that the overall diagnostic yield from NPAs was superior. In a related study, Sung et al[39] compared NPA and NS specimen collection methods to results from three different assays: Immunofluorescence test (IF), viral culture (VC) and more importantly, PCR. Using the Cohen Kappa test (95% Confidence Interval, 0.6-0.8 meaning high agreement) there was a high concordance between NS and NPA for PCR tests involving Adenovirus, Influenza A, Influenza B, Parainfluenza and RSV. Do et al. [38] recommended the combination of NS and TS for generally sampling .

In the area of diagnosis, current methods employed to diagnose and detect respiratory pathogens include various polymerase chain reaction (PCR) assays[40,41,42] and antigen tests using different fluorescence and enzyme-linked immunoassays [43]. Though enzyme-linked immunoassays are easy to use and suited for use especially in the field and in cases where many samples are to be tested for pathogens, it was not available for this study.Rapid lateral flow immunoassays were used. They are however unreliable in the sense that: they

sometimes fail to detect pathogens and they only indicate the presence or absence of a pathogen but not the genetic sequence, concentration or other information that may be relevant to clinicians or researchers. PCR, on the other hand, is currently used not only as a confirmatory assay but also as a diagnostic test. It is economical in terms of time and resources, but the several methods for real-time PCR assay design have very low sensitivities for many clinical applications[44], this is due mainly to the probe design and quality of primers. Also, in resource-poor settings PCR is not applicable in many areas due to resource limitations and low technical capabilities, but the rapid automated PCR system used in this study, is easy to use, runs for a shorter period and reduces turn-over time. However, unlike other PCR assays, the thermocycler processes one sample at a time and is not ideal for handling bulk samples during outbreak situations and is also expensive to run, as each pouch cost about 100 US dollars, making 120 pouches worth 12,000 US dollars, excluding value of operator time.

Generally, there were detections of bacterial pathogens on both molecular platforms used, with pathogens: *Streptococcus pneumonia, Klebsiella pneumonia*, *Chlamydophila pneumonia* and *Mycoplasma pneumonia, four* bacterial causative agents associated with atypical and community-acquired pneumonia[45,46]; *Haemophilus influenzae,* which exist both as a commensal in adults[47,48] and as a serious source of morbidity in children, causing meningitis, pneumonia and bacteremia[47,48]; *Moraxella catarrhalis which is* responsible for certain bronchopulmonary infections in adults and otitis media and sinusitis in children[49,50] . These results are consistent with other studies that have also indicated the role bacteria play in infections of the nasal sinuses and pharynx leading to rhinosinusitis and pharyngitis, epiglottitis and laryngotracheitis[9]. In addition pneumonia disease alone can kill over 1.6 million people each year, with vast a majority of its victims coming from the world's poorest countries such as Sierra Leone. [51]. This study also indicates high bacterial activity in the causation of respiratory infections in Bo, Sierra Leone.

Though computational methods, including gene resequencing, microarray scan analysis, and comparisons against data found on GeneBank by alignment, do confirm the presence of certain pathogens; biological methods used in this study can be a cause for the contrast in detection and identification of emerging and infectious respiratory agents. The storage of the nasopharyngeal swab samples is a possible source of discrepancy. The FTA cards, protein

saver types, may have been better in stabilizing DNA and protein-rich samples and less efficient in preserving RNA-based microorganisms, in this case respiratory viruses, that were stored on to these cards. Additionally, spotting preparations for these samples may have diluted them to a degree that lowered sensitivity. Sample storage may account for the absence of RNA-based viruses during detection using RPM-Flu v.3.1. Nonetheless, when comparing diagnostic capabilities, FilmArray is simpler and thus faster, but RPM-Flu carries greater detection resolution and is capable of detecting and identifying a broader range of pathogens while differentially separating apart notable strains and sequencing divergences for both bacteria and viruses.

4.4 CONCLUSION

The high syndromic prevalence of respiratory infections suggests that these type of infections constitute a standard non-malarial condition in Bo Sierra Leone. Further, both viruses and bacteria remain important etiologic agents of respiratory infections. Further studies are required to compare the diagnostic capabilities of the multiplex PCR system and RPM, where discrepant results were observed.

4.5 REFERENCES

1. Kebede S, Conteh IN, Steffen CA, et al. Establishing a national influenza sentinel surveillance system in a limited resource setting, experience of Sierra Leone. Health Res Policy Syst [Internet] 2013;11:22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3694480&tool=pmcentrez&rendertype=abstract

2. Breu F, Guggenbichler S, Wollmann J. World Health Statistics 2013. Vasa [Internet] 2013;168. Available from: http://medcontent.metapress.com/index/A65RM03P4874243N.pdf

3. Torlesse H, Wurie IM, Hodges M. The use of immunochromatography test cards in the diagnosis of hepatitis B surface antigen among pregnant women in West Africa. Br J Biomed Sci 1997;54:256–9.

4. Wurie IM, Wurie AT, Gevao SM. Sero-prevalence of hepatitis B virus among middle to high socio-economic antenatal population in Sierra Leone. West Afr J Med [Internet] 2005 [cited 2014 May 17];24(1):18–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15909704

5. Koroma JB, Bangura MM, Hodges MH, Bah MS, Zhang Y, Bockarie MJ. Lymphatic filariasis mapping by Immunochromatographic Test cards and baseline microfilaria survey prior to mass drug administration in Sierra Leone. Parasit. Vectors. 2012;5:10.

6. Hodges ME, Koroma JB, Sonnie M, Kennedy N, Cotter E, Macarthur C. Neglected tropical disease control in post-war Sierra Leone using the Onchocerciasis Control Programme as a platform. Int. Health. 2011;3:69–74.

7. World Health Organization, Organization WH. World Health Statistics 2008 [Internet]. 2008. Available from: http://www.who.int/whosis/whostat/EN_WHS08_TOCintro.pdf

8. Bezerra PGM, Duarte M do CMB, Britto MCA, Correia JB. Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. J Pediatr (Rio J) [Internet] [cited 2014 Jun 6];87(5):461; author reply 462. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22011744

9. Liu. P, Dasaraju V, Liu C. Infections of the Respiratory System [Internet]. In: Medical Microbiology. 1996. Available from: http://www.ncbi.nlm.nih.gov/books/NBK8142/

10. Barbara Bannister, Stephen Gillespie JJ. Infection: Microbiology and Management. 3rd Editio. Wiley-Blackwell; 2009.

11. Treebupachatsakul P, Tiengrim S, Thamlikitkul V. Upper respiratory tract infection in Thai adults: prevalence and prediction of bacterial causes, and effectiveness of using clinical practice guidelines. J Med Assoc Thai 2006;89:1178–86.

12. Murdoch DR. Molecular genetic methods in the diagnosis of lower respiratory tract infections. APMIS [Internet] 2004;112:713–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15638835

13. Guthrie R. Community-acquired lower respiratory tract infections: etiology and treatment. Chest 2001;120:2021–34.

14. Bharaj P, Sullender WM, Kabra SK, et al. Respiratory viral infections detected by multiplex PCR among pediatric patients with lower respiratory tract infections seen at an urban hospital in Delhi from 2005 to 2007. Virol J 2009;6:89.

15. Martin SA, Pence BD, Woods JA. Exercise and respiratory tract viral infections. Exerc Sport Sci Rev 2009;37:157–64.

16. Pavia AT. Viral infections of the lower respiratory tract: Old viruses, new viruses, and the role of diagnosis. Clin Infect Dis 2011;52.

17. Steffen C, Debellut F, Gessner BD, et al. Improving influenza surveillance in sub-Saharan Africa. Bull World Health Organ [Internet] 2012;90:301–5. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3324873&tool=pmcentrez&rendertype=abstract

18. Nair H, Brooks WA, Katz M, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet [Internet] 2010;378:1917–30. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22078723

19. P.G.M. B, M.C.A. B, J.B. C, et al. Viral and atypical bacterial detection in acute respiratory infection in children under five years [Internet]. PLoS One. 2011;6. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=2011227043

20. Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. Lancet Infect. Dis. 2002;2:25–32.

21. UN. The Millennium Goals Report. – 35M. New York:

22. Poritz MA, Blaschke AJ, Byington CL, et al. Filmarray, an automated nested multiplex PCR system for multi-pathogen detection: Development and application to respiratory tract infection. PLoS One 2011;6.

23. N.E. B. The FilmArray respiratory panel: An automated, broadly multiplexed molecular test for the rapid and accurate detection of respiratory pathogens [Internet]. Expert Rev. Mol. Diagn. 2013;13:779–88. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed11&NEWS=N&AN=2013708257

24. Kristen Kanack, Kevin Bourzac, Samuel Richards, Ranae Lems, Peta-Gaye Klenk, Kristen Bloch, Whitney Oswald and BL. Detection of Bacterial Targets by a Multiplexed Respiratory Panel: Bordetella pertussis, Chlamydophila pneumoniae, Mycoplasma pneumoniae [Internet]. [cited 2014 Jun 1];Available from: http://filmarray.com/assets/pdf/Detection-of-Bacterial-Targets-by-a-Multiplexed-Respiratory-Panel.pdf

25. Malanoski AP, Lin B, Stenger DA. A model of base-call resolution on broad-spectrum pathogen detection resequencing DNA microarrays. Nucleic Acids Res 2008;36:3194–201.

26. Lin B, Blaney KM, Malanoski AP, et al. Using a resequencing microarray as a multiple respiratory pathogen detection assay. J Clin Microbiol 2007;45:443–52.

27. Ansumana R. Use of fast technology for the analysis of nucleic acids to store ribonucleic acid extracts at elevated temperatures. Int J Collab Res Intern Medi 2011;3(3):261.

28. Mäkelä MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. J Clin Microbiol 1998;36:539–42.

29. Wat D. The common cold: A review of the literature. Eur. J. Intern. Med. 2004;15:79–88.

30. Boeckh M, Geballe AP. Cytomegalovirus: Pathogen, paradigm, and puzzle. J. Clin. Invest. 2011;121:1673–80.

31. Denoeud L, Turbelin C, Ansart S, Valleron AJ, Flahault A, Carrat F. Predicting pneumonia and influenza mortality from morbidity data. PLoS One 2007;2.

32. Denoeud L, Turbelin C, Ansart S, Valleron AJ, Flahault A, Carrat F. Predicting pneumonia and influenza mortality from morbidity data. PLoS One 2007;2(5).

33. Simonsen L, Reichert TA, Viboud C, Blackwelder WC, Taylor RJ, Miller MA. Impact of influenza vaccination on seasonal mortality in the US elderly population. Arch Intern Med 2005;165:265–72.

34. Simonsen L, Taylor RJ, Viboud C, Miller MA, Jackson LA. Mortality benefits of influenza vaccination in elderly people: an ongoing controversy. Lancet Infect. Dis. 2007;7:658–66.

35. Covalciuc KA, Webb KH, Carlson CA. Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods. J Clin Microbiol 1999;37:3971–4.

36. McIntosh K, Halonen P, Ruuskanen O. Report of a workshop on respiratory viral infections: epidemiology, diagnosis, treatment, and prevention. Clin Infect Dis 1993;16:151–64.

37. Schmid ML, Kudesia G, Wake S, Read RC. Prospective comparative study of culture specimens and methods in diagnosing influenza in adults. BMJ [Internet] 1998 [cited 2014 Aug 15];316(7127):275. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2665515&tool=pmcentrez&rendertype=abstract

38. Do AHL, van Doorn HR, Nghiem MN, et al. Viral etiologies of acute respiratory infections among hospitalized vietnamese children in Ho Chi Minh City, 2004-2008. PLoS One 2011;6.

39. Sung RYT, Chan PKS, Choi KC, et al. Comparative study of nasopharyngeal aspirate and nasal swab specimens for diagnosis of acute viral respiratory infection. J Clin Microbiol 2008;46:3073–6.

40. Payungporn S, Phakdeewirot P, Chutinimitkul S, et al. Single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) for influenza A virus subtype H5N1 detection. Viral Immunol 2004;17:588–93.

41. Lee MS, Chang PC, Shien JH, Cheng MC, Shieh HK. Identification and subtyping of avian influenza viruses by reverse transcription-PCR. J Virol Methods 2001;97:13–22.

42. Lau LT, Banks J, Aherne R, et al. Nucleic acid sequence-based amplification methods to detect avian influenza virus. Biochem Biophys Res Commun 2004;313:336–42.

43. Hien TT, de Jong M, Farrar J. Avian influenza--a challenge to global health care structures. N Engl J Med [Internet] 2004 [cited 2014 Aug 16];351(23):2363–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15575048

44. Lemmon GH, Gardner SN. Predicting the sensitivity and specificity of published real-time PCR assays. Ann Clin Microbiol Antimicrob 2008;7:18.

45. Jones RN, Jacobs MR, Sader HS. Evolving trends in Streptococcus pneumoniae resistance: Implications for therapy of community-acquired bacterial pneumonia. Int. J. Antimicrob. Agents. 2010;36:197–204.

46. Prince SE, Dominger KA, Cunha BA, Klein NC. Klebsiella pneumoniae pneumonia. Hear Lung J Acute Crit Care 1997;26:413–7.

47. King P. Haemophilus influenzae and the lung (Haemophilus and the lung). Clin. Transl. Med. 2012;1:10.

48. Agrawal A, Murphy TF. Haemophilus influenzae infections in the H. influenzae type b conjugate vaccine era. J. Clin. Microbiol. 2011;49:3728–32.

49. Nowak-Sadzikowska J, Heczko PB. Moraxella catarrhalis as an important etiological factor in infection of the lower bronchical tree. Pneumonol. Alergol. Pol. 1994;62:530–2.

50. Paykel JM. Moraxella (Branhamella) catarrhalis infections. Prim. Care Update Ob. Gyns. 2002;9:33–5.

51. APPG. ImprovIng global health by preventIng pneumococcal dIsease:Report from the All-Party Parliamentary Group on Pneumococcal Disease Prevention in the Developing World. LONDON, UK: 2007.

CHAPTER FIVE

COMMON FEBRILE ILLNESSES IN SIERRA LEONE:REEMERGENCE OF CHIKUNGUNYA VIRUS IN BO, SIERRA LEONE

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5.1 ABSTRACT

Background

Outbreaks of infection with chikungunya virus (CHIKV), an alphavirus that is transmitted by bites of infected *Aedes* spp. mosquitoes, were frequent in sub-Saharan Africa and southern and Southeast Asia during the 1950s–1970s, but the infection largely disappeared in the 1980s; only sporadic cases were observed [1]. The virus reemerged in the early 2000s.

Objective

The main goal of the study was to investigate the incidence and/prevalence of non-malarial febrile illnesses such as Chikungunya in Bo Sierra Leone and to determine the geographical distribution of the infection using a crowdsourcing platform, ushahidi.

Methods

A tiered analysis approach was used. First, all specimens from febrile study participants were tested for ≈12 infections with various pathogens, including CHIKV, by commercially available test kits. Specimens that showed negative results in this first round of testing were further tested by using cultures, multiplex PCR, and resequencing pathogen microarrays.

Results

Using person-time rate at 95% confidence interval (CI) per 1000 people per year, the incidence of Chikungunya within the cohort was calculated to be 573.7. The prevalence was 46.04%; 95%CI: 43.45- 48.66% with ages ranging from 6 years to 85 years; 344(53.25%; 95%CI: 49.32-57.14%) were female patients. Of these 646 ChikV patients, 370 (57.3.0%) reported arthralgia, 390 (60.4%) chills, and 355 (55.0%) headaches. Co-infections were common; 69 (10.68%; 95%CI 8.5-13.3%) were co-infected with malaria, 37 (5.7%; 95% CI: 4.2-7.8%) with HIV, 36 (5.6%95% CI: 4.1-7.6%) with viral hepatitis and smaller numbers with tuberculosis, typhoid, and syphilis. Twelve CHIKV-positive samples were also positive for dengue.

Conclusion

The results suggest that an outbreak of chikungunya virus occurred in Sierra Leone. Because Mercy Hospital serves only a relatively small proportion of the residents of Bo, the cases detected likely represent only a small fraction of the total cases that have occurred. Further study will be required to document the extent of the outbreak.

5.1. INTRODUCTION

Outbreaks of infection with chikungunya virus (CHIKV), an alphavirus that is transmitted by bites of infected *Aedes* spp. mosquitoes, were frequent in sub-Saharan Africa and southern and Southeast Asia during the 1950s–1970s, but the infection largely disappeared in the 1980s; only sporadic cases were observed [1]. The virus reemerged in the early 2000s; major outbreaks were reported in Kenya, some island nations in the Indian Ocean, and several countries in Asia.

The primary symptoms of CHIKV infection are high fever (>38.5°C [102°F]) and severe pain in the distal joints of the extremities or the lumbar spine. A maculopapular rash, sensorineural impairment, severe headache, and other nonspecific symptoms may also occur. Symptoms usually resolve within 1–2 weeks after onset of fever, but for a sizeable proportion of patients, arthralgia and arthritis become chronic and pain persists for years [2,3]

A nationwide serosurvey in Sierra Leone in 1972 detected cases of CHIKV infection throughout the country [4], but we are not aware of any cases reported since the mid-1970s. Two recent developments made reemergence appear imminent. First, outbreaks of reemerging CHIKV have been reported in neighboring Guinea [5] and in Senegal [6] Second, recent yellow fever cases in Sierra Leone have shown that *Aedes* spp. mosquito–borne infections are common[7]. Thus, it was not surprising when we conducted an infectious disease surveillance study in July 2012 in the city of Bo, in Southern Province, Sierra Leone, that we detected possible chikungunya virus infections.

The main goal of the study was to investigate the incidence and/prevalence of non-malarial febrile illnesses such as Chikungunya in Bo Sierra Leone and to determine the geographical distribution of the infection using a crowdsourcing platform, ushahidi.

5.2 METHODS

On July 7, 2012, a one-year prospective cohort study at the Mercy Hospital Research Laboratory (MHRL) in Bo, Sierra Leone, was initiated to identify the diversity of pathogens causing febrile illness in the city. A tiered analysis approach was used. First, all specimens from febrile study participants were tested for ≈12 infections with various pathogens, including CHIKV, by commercially available test kits. Specimens that showed negative results in this first round of testing were further tested by using cultures, multiplex PCR, and resequencing pathogen microarrays. The research protocol was approved by Njala University, George Mason University, the Liverpool School of Tropical Medicine, the US Naval Research Laboratory, and the Sierra Leone Ethics and Scientific Review Committee.

5.2.1 Tier 1 Analyses

During July 7, 2012–July 7,2013, a first-tier lateral flow immunoassay (LFI) tests of blood samples were conducted from outpatients ≥5 years of age who had been clinically examined at the hospital, were found to have febrile illness, and consented to having blood drawn for laboratory testing. LFI test kits (SD Bioline; Standard Diagnostics, Inc., Seoul, South Korea) were used for diagnosis of IgM against CHIKV; IgG and IgM against dengue virus and hepatitis A virus; hepatitis B virus surface antigen, hepatitis C virus, HIV-1/2, and antibodies against these viruses; and IgG and IgM against *Leptospira* spp., *Salmonella enterica* Serovar typhi, and syphilis.

5.2.2 Tier 2 analyses

Tier 2 analyses were comprised of semi-nested PCR and 6 PCRs targeting different regions of the viral genome, including alphavirus consensus PCR, flavivirus consensus PCR, filovirus consensus PCR, arenavirus consensus PCR. For the semi-nested PCR the method used was adapted from Rianthavorn[8].

In the initial PCR, DVRChkF 50-ACCGGCGTC TACCCATTCATGT-30 (nt 10237–10258)18 and CU3-CHIKR 50-TCGCTRCAGCACACRGCACC-30 (nt 10741–10760) were used as forward primer and reverse primer, respectively. The Roche High-Fidelity

Fast-Start PCR kit (Roche Diagnostics, Indianapolis, IN, USA) was used together with the Applied Biosystems Gene Amp **9700** thermocycler (Perkin Elmer, Norwalk, CT. USA) and the Bio-Rad MJ Mini Thermal cycler (Bio-Rad Laboratories, Hercules, CA). The cycling conditions for reverse transcription were; 25°C for 10 minutes; 50°C for 50 minutes, 85°C for 5 minutes and 4°C forever. This was then followed by the first PCR with cycling conditions: 95°C for 2 minutes for denaturation, followed by 40 amplification cycles 95°C for 30 seconds for denaturation, 55°C for 30 seconds for primer annealing, 72°C for extension and 95°C for 2 minutes for final extension. Then, the semi-nested PCR was performed by using CU1CHIKF 50-GCATCAGCTAAGCTCCGCGTC-30 (nt 10378–10398) as an inner forward primer. Gel Electrophoresis was done by the Flash gel DNA System (**Lonza Group Ltd** Basel, Switzerland).

5.2.3 Tier 3 analyses

The resequencing pathogen microarray system RPM-TEI v. 1.0 described previously by Leski et al[9,10] was used in this study to rule out O'nyong-nyong and Dengue, viruses that may cause symptoms similar to those caused by chikungunya virus. In addition the new Resequencing Pathogen Microarray, the IVDC4-03, with tiles for Chikungunya was also used.

Additionally, for all chikungunya genes on genebank, the evolutionary history was inferred using the Minimum Evolution method[11]. The optimal tree with the sum of branch length = 0.39778494 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method[12] and are in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm[13] at a search level of 1. The Neighbor-joining algorithm[14] was used to generate the initial tree. The analysis involved 129 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 11338 positions in the final dataset. Evolutionary analyses were conducted in MEGA5[15].

5.3 RESULTS

Using person-time rate at 95% confidence interval (CI) per 1000 people per year, the incidence of Chikungunya within the cohort was calculated to be 573.7.

More than half of the cases tested during the first month of the surveillance program were positive by LFI for CHIKV. Thus, we notified the Sierra Leone Ministry of Health and Sanitation of a possible CHIKV outbreak. By June 30, 2013, 646(46.04%; 95%CI: 43.45- 48.66%) febrile patients were positive by LFI for CHIKV (Figure 5.1). Ages of the 619 CHIKV IgM-positive patients ranged from 6 years to 85 years; 344(53.25%; 95%CI: 49.32-57.14%) were female patients.

Of these 646 patients, 370 (57.3.0%) reported arthralgia, 390 (60.4%) chills, and 355 (55.0%) headaches. Co-infections were common; 69 (10.68%; 95%CI 8.5-13.3%) were co-infected with malaria, 37 (5.7%; 95% CI: 4.2-7.8%) with HIV, 36 (5.6%95% CI: 4.1-7.6%) with viral hepatitis and smaller numbers with tuberculosis, typhoid, and syphilis. Twelve CHIKV-positive samples were also positive for dengue.

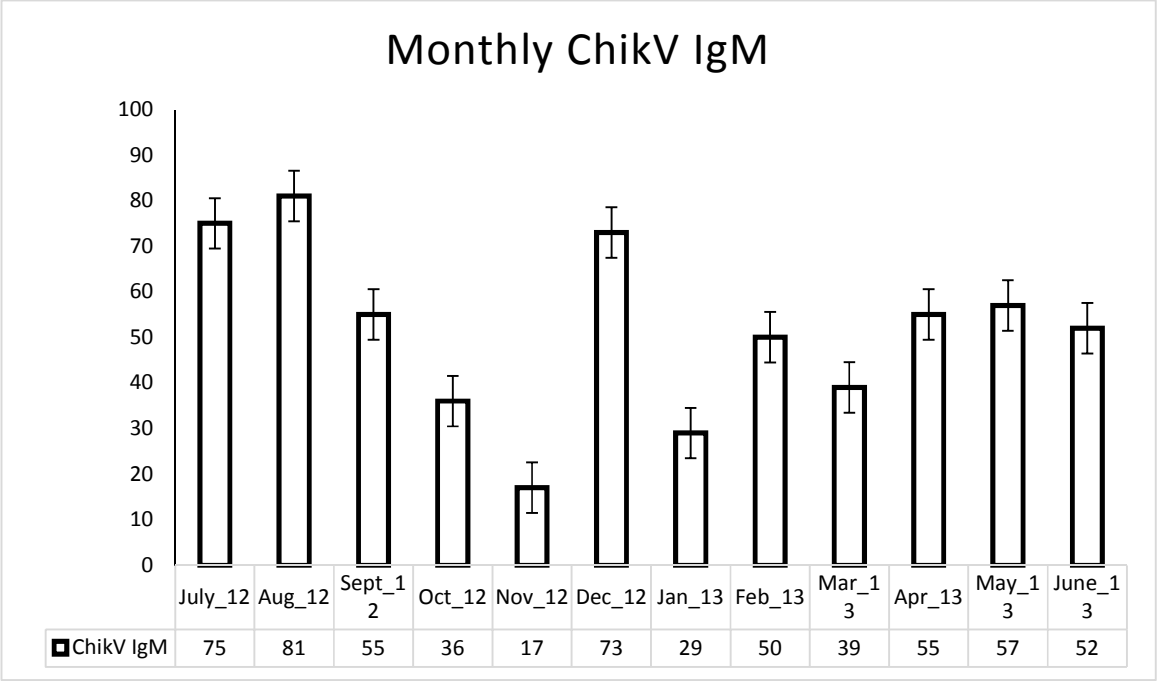


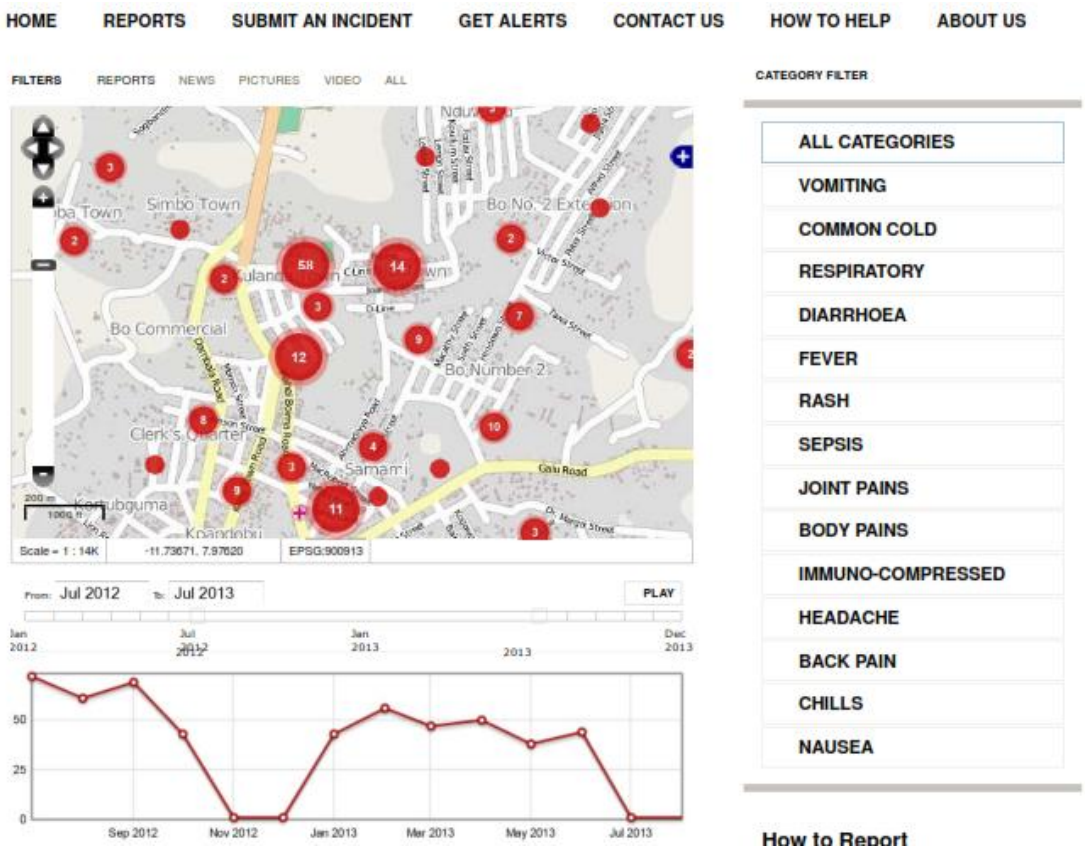
Figure 5.1: Monthly number of IgM-positive chikungunya virus test results at Mercy Hospital Research Laboratory, Bo, Sierra Leone, July 7, 2012–June 30, 2013

Most patients reported that they had sought medical care within several days after the onset of their febrile illnesses. Levels of IgM against CHIKV are usually detectable by immunochromatographic methods within a few days after infection and persist for ≈3–4 months [1,2] The LFI test kits for CHIKV were reported by the manufacturer to have a sensitivity of 97.1% and a specificity of 91.1% compared with those of ELISA [16]. An independent evaluation found a sensitivity of 50.8% and a specificity of 89.2% for the kits; sensitivity ranged from 40.9% 1–5 days after onset of illness to 65.4% 16–20 days after onset [17].Specificity decreases after the first week [18,19].

On July 28, an Ushahidi-based website (www.ushahidi.com) to compile case reports was launched. Details about the patients who were positive for CHIKV were uploaded to a website (www.mhrlsl.com/GIA/ushahidi) and, if possible, were geolocated on an open

street map (www.openstreetmap.org) that linked to a map of Bo created previously for health research purposes [20]. The map showed the geographical distribution of Chikungunya within the city of Bo (Fig 5.2). However, because the sample was not population-based because Mercy Hospital is 1 of several hospitals serving Bo, a city-wide attack rate could not be determined.

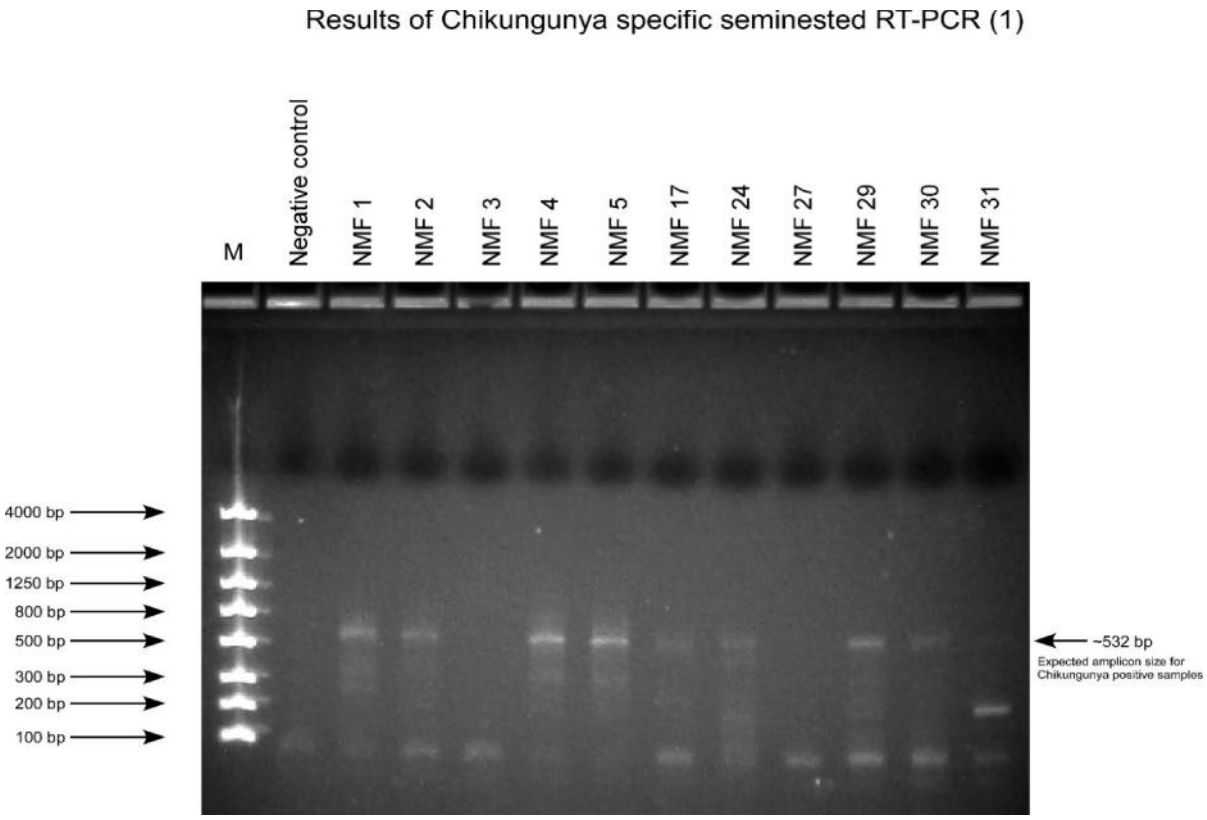
Figure 5.2: Residence locations for IgM-positive cases of infection with chikungunya virus, Bo, Sierra Leone, July, 2012–July 2013.



Results from the Semi-nested PCR is shown below (Fig 5.3). 8 out of 15 samples tested by semi-nested PCR were positive and 2 of the 8 that were positive by Semi-nested PCR were IgM negative for ChikV. However the 18 samples tested with the other 6 PCRs targeting

different regions of the viral genome, including alphavirus consensus PCR, flavivirus consensus PCR, filovirus consensus PCR, arenavirus consensus PCR yielded negative results. The RPM TEI lacks tiles for chikungunya but results were negative for onyong nyong and dengue which may produce similar symptoms as Chikungunya.

Fig 5.3: Results of Chikungunya Specific Seminested RT-PCR



The negative results from the other PCRs could be possibly because of genetic sequence variation from well-characterized strains or because of the timing of specimen collections. Viral loads for humans with CHIKV infection decrease after the second day of symptoms, and viral titers may be low after the fifth day [18,19]. Because CHIKV nucleic acids are only detectable in serum for a few days, reverse transcription PCR results are often discordant with those of serologic (IgM and IgG) assays. Confirmation that an outbreak occurred is dependent on isolation of the virus, followed by molecular characterization, full-genome sequencing, and phylogenetic mapping. Due to funding limitations sequencing of the virus was not done, but all genes on GeneBank for Chikungunya were extracted and a phylogenetic tree drawn with Mega 5.1(Fig 5.4)

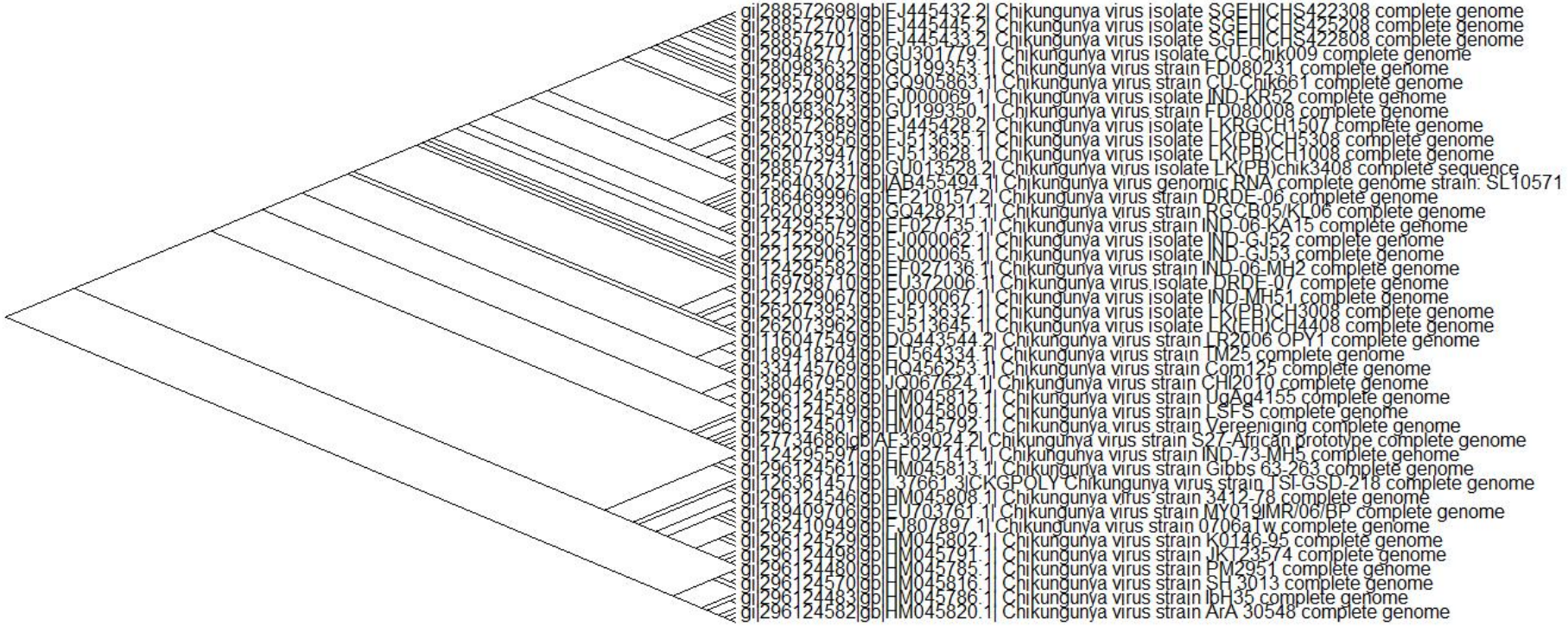


Figure 5.4: Evolutionary relationships of taxa

5.4 CONCLUSIONS

The results suggest that an outbreak of chikungunya virus occurred in Sierra Leone. The exact time of the reemergence of this virus cannot be pinpointed, but retrospective analyses of outpatient charts suggested that, on the basis of syndromic criteria, the first cases occurred in January 2012 and the outbreak peaked during the rainy season in 2012. Other outbreaks reported in central and west Africa have also occurred during the rainy season, which is typical for *Aedes* spp. mosquito–borne infections[6,21,22] . Because Mercy Hospital serves only a relatively small proportion of the residents of Bo, the cases detected likely represent only a small fraction of the total cases that have occurred. Further study will be required to confirm the laboratory results and, if further investigation is warranted, to document the extent of the outbreak.

5.5 References

1. Ng LC, Hapuarachchi HC. Tracing the path of Chikungunya virus-Evolution and adaptation. Infect. Genet. Evol. 2010;10:876–85.

2. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: A re-emerging virus. In: The Lancet. 2012. p. 662–71.

3. Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clin Infect Dis 2009;49:942–8.

4. Mouchet J RY. Mouchet J, Robin Y. Serological and entomological survey on yellow fever in Sierra Leone: report on a mission May 16–June 7, 1972. 1973.

5. Jentes ES, Robinson J, Johnson BW, et al. Acute arboviral infections in Guinea, West Africa, 2006. Am J Trop Med Hyg 2010;83:388–94.

6. Thonnon J, Spiegel A, Diallo M, Diallo A, Fontenille D. [Chikungunya virus outbreak in Senegal in 1996 and 1997]. Bull Soc Pathol Exot [Internet] 1999 [cited 2014 May 29];92(2):79–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10399593

7. WHO. Outbreak news. Yellow fever, Sierra Leone. Wkly Epidemiol Rec [Internet] 2011 [cited 2014 May 29];86(12):101–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21442796

8. Rianthavorn P, Prianantathavorn K, Wuttirattanakowit N, Theamboonlers A, Poovorawan Y. An outbreak of chikungunya in southern Thailand from 2008 to 2009 caused by African strains with A226V mutation. Int J Infect Dis [Internet] 2010 [cited 2014 Sep 26];14 Suppl 3:e161–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20417142

9. Leski TA, Lin B, Malanoski AP, et al. Testing and validation of high density resequencing microarray for broad range biothreat agents detection. PLoS One 2009;4.

10. Leski TA, Ansumana R, Malanoski AP, et al. Leapfrog diagnostics: Demonstration of a broad spectrum pathogen identification platform in a resource-limited setting. Heal. Res. Policy Syst. 2012;10:22.

11. Rzhetsky A, Nei M. A Simple Method for Estimating and Testing Minimum-Evolution Trees. Mol Biol Evol [Internet] 1992;9:945–67. Available from: <Go to ISI>://A1992JK45600014

12. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci U S A 2004;101:11030–5.

13. S. NM and K. Molecular Evolution and Phylogenetics. New York: Oxford University Press; 2004.

14. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406–25.

15. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731–9.

16. Standard Diagnostics ICI. Standard Diagnostics, Inc. Chikungunya IgM [Internet]. 2012;Available from: http://www.standardia.com/html_e/mn03/mn03_01_00.asp?intId=119

17. Kosasih H, Widjaja S, Surya E, et al. Evaluation of two IgM rapid immunochromatographic tests during circulation of Asian lineage Chikungunya virus. Southeast Asian J Trop Med Public Health [Internet] 2012;43:55–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23082554

18. Ray P, Ratagiri VH, Kabra SK, et al. Chikungunya infection in India: Results of a prospective hospital based multi-centric study. PLoS One 2012;7.

19. Laurent P, Le Roux K, Grivard P, Bertil G, Naze F, Picard M. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify chikungunya virus. Clin Chem 2007;53:1408–14.

20. Ansumana R, Malanoski AP, Bockarie AS, et al. Enabling methods for community health mapping in developing countries. Int J Health Geogr 2010;9:56.

21. Demanou M, Antonio-Nkondjio C, Ngapana E, et al. Chikungunya outbreak in a rural area of Western Cameroon in 2006: A retrospective serological and entomological survey. BMC Res Notes 2010;3:128.

22. Caron M, Paupy C, Grard G, et al. Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. Clin Infect Dis [Internet] 2012 [cited 2014 May 29];55(6):e45–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22670036

CHAPTER SIX

COMMON FEBRILE ILLNESSES IN SIERRA LEONE: HIV INFECTION

6.1 ABSTRACT

Objective(s): The goal of this study was to investigate the prevalence and incidence of HIV in a cohort of subjects within the catchment of Mercy Hospital in Bo Sierra Leone.

Design: A cohort of 1403 subjects were recruited randomly from the catchment population of Mercy Hospital and followed for a period of one year with laboratory tests done when subjects become febrile.

Methods: All subjects got tested with an SD Bioline 4th Generation HIV Ag/Ab Combo lateral flow immunoassay (Standard Diagnostics Inc. Kyonggi-do, Korea). The test kit is a qualitative test for detecting HIV p24 antigen and antibodies (IgG, IgM and IgA) specific to HIV-1 including subtype-O and/or HIV-2 in human serum, plasma or whole blood. Other tests for syphilis, malaria, HBsAg, HCV, HAV, were done. Patient blood chemistry tests were done using CHEM8+ cartridges of the Abbott Point of Care Chemistry analyzer, I`-STAT 300 (Abbott Laboratories. Abbott Park, IL USA).

Results: A person-time rate of 102.4 HIV cases at 95% CI per 1000 people per year were determined in Bo Sierra Leone. The prevalence of HIV was 9.34 %(100/1403); 61% were females and 39% males. Of those that were having HIV, 10.69 %(14/131) were co-infected with Hepatitis B; 3.1 %(4/131) were having syphilis; 1.5 %(2/131) were having Hepatitis C virus. Blood chemistry of 32 HIV- patients were assessed, 15(46.88%) had hemoglobin values below 12g/dl with a median Hb value of 10.90g/dl and 6(18.75%) of hematocrit values were critically low (≤27%PCV).

Conclusions: This study provides additional evidence that the HIV epidemic is a serious problem in the city of Bo Sierra Leone. There is a need for more independent studies on the prevalence of HIV in Sierra Leone and to improve the diet of those affected by HIV.

KEYWORDS: HIV, COMMON, FEBRILE, ILLNESS, BO, SIERRA LEONE

6.2 INTRODUCTION

Human immune-deficiency virus (HIV) and the Acquired Immune Deficiency Syndrome (AIDS) which results from HIV infection, remain among the most dreaded diseases worldwide. Twenty-one countries of the world with the highest rates of HIV are in sub-Saharan Africa; eight of them have more than one million people living with HIV and overall, HIV has infected more than 40 million Africans since the start of the epidemic; 22 million of which have died. [1]. In 2012, there were 2.3 (1.9–2.7) million new HIV infections globally, depicting a 33% decline in incident cases 3.4 (3.1–3.7) million in 2001 and there was a concomitant decrease in AIDS deaths in 1.6 (1.4–1.9) million AIDS deaths in 2012, compared to 2.3 (2.1–2.6) million deaths in 2005[2].

In Sierra Leone, since 1987 when the first cases of HIV/AIDS were identified in Bo and Kenema Districts, there has been an increase in the number of persons living with HIV/AIDS [3]. The country's HIV epidemic is mixed, generalized and heterogeneous as the infection cuts across all sectors and different strata of the population through varied transmission dynamics[4]. National seroprevalence of HIV peaked in 2005 and stabilized in 2007 and adults with HIV were estimated at 1.5% , and pregnant women attending antenatal clinics were 3.2% prevalent[4]. However, there is no concordance between the National HIV prevalence figures in Sierra Leone and independent research studies carried out in the country.

In 2002, it was reported that about 7% of women receiving antenatal care in Freetown had AIDS[5]. In the same year, country-wide, 45,000 persons were reported to be having HIV and in 2005; 75,000 were with HIV[6]. The percentage increase in three years was 66.7%[3] but reported national prevalence has been static since 2005. Students have done most studies that have been done on HIV in Sierra Leone and they remain unpublished. However, published data on HIV are not consistent with national figures.

In Bo, seroprevalence reported by the Ministry of Health and Sanitation(MOHS) in 2005 was 1.9%, and higher percentage of females were affected than males[7]. Bhoobun et al[8] working on facilitators and barriers related to voluntary counseling and testing for HIV

among young adults in Bo reported that over two-thirds of young adults have never been tested for HIV and over two-thirds of those not tested previously were willing to try in the near future, but report fears about family/partner rejection, job loss, and other potential consequences of testing and a strong desire for robust testing privacy. There are seemingly more persons positive for HIV that are yet to be tested.

This study investigated the prevalence and/ incidence of HIV in a cohort of subjects within the catchment of Mercy Hospital in Bo Sierra Leone.

6.3 METHODS

A cohort of 1403 subjects were recruited randomly from the catchment population of Mercy Hospital and followed for a period of one year. The selections were prefaced by a syndromic surveillance survey described previously[9].

All subjects were tested with an SD Bioline 4th Generation HIV Ag/Ab Combo lateral flow immunoassay (Standard Diagnostics Inc. Kyonggi-do, Korea) which is a rapid, qualitative test for the detection of HIV p24 antigen and antibodies to isotypes IgG, IgM and IgAspecific to HIV-1 including subtype-O and/or HIV-2 simultaneously in human serum, plasma or whole blood[10]. Positive HIV tests were redone with the SD Bioline Kit and the Alere Determine™ HIV-1/2(Alere Medical Co Ltd. Matsudo-shi, Chiba, Japan). Other tests for syphilis, malaria, HBsAg, HCV, HAV, were done. Patient blood chemistry tests were done using the Abbott Point of Care Chemistry analyser, i-STAT 300 (Abbott Laboratories. Abbott Park, IL USA), with the CHEM8+ cartridges.

6.4 RESULTS AND DISCUSSION

The prevalence of HIV in Bo was 9.7% % (95%CI: 8.2-11.4)(136/1403). Of those that were having HIV, 61% were females and 10.3 %(14/136) were coinfected with Hepatitis B; 3.0 %(4/136) were having syphilis, 1.5 %(2/136) were having Hepatitis C virus. A person-time rate of 102.4 HIV at 95% CI per 1000 people per year was determined in Bo Sierra Leone.

Blood chemistry of 32 HIV-positive patients were assessed(Table 6.1), 15(46.9%) had hemoglobin(Hb) values below the reference range of(12-17 g/dL);mean Hb values was 12.0(± 3.35SD) and median value was 11.8g/dl; only 9(28.1%) of hematocrit values were within the reference range(38-51 %PCV), 6(18.8%) were critically low(≤27%PCV) and the average %PCV for critically low values was 21.7(±1.96). 11(34.4%) had glucose above the reference range of (70-105mg/dl) and a mean of 143.5± 50.6mg/dL; 30(93.8%) had ionized calcium (iCa) below the reference range of 1.2 -1.3mmol/L with a mean iCa of 1.1 mmol/L but 2(6.3%) had critically low iCa (≤0.7 mmol/L). 5(15.6%) patients had creatinine values above the critical value (≥2.0 mg/dL), and the average of the critically high values was 2.6(±0.7SD). For potassium ions, 2(6.3%) had critically high values (≥6.5 mmol/L) ; 2(6.3%) also had critically high values (≥120 mmol/L) of Chloride ions and 1(3.1%) had critically low value (≤15 mmol/L) of total carbon dioxide (TC02). All other values were within the critical values with the following averages: urea nitrogen (BUN) was 13.4(±12.5) mg/dL; sodium ion (Na) 134.84(±6.3) mmol/L; anionic gap (AnGap) was 14.7(±4.1) mmol/L.

This study demonstrates that the HIV epidemic remains a serious problem in Bo Sierra Leone. The prevalence of 9.7% in this study is consistent with findings from other independent studies carried out in Sierra Leone. In Kenema, one of the first cities where HIV was initially reported in Sierra Leone, Kouyoumdjian[11] reported HIV prevalence of 12.6% in women specifically accessing voluntary counselling and testing (VCT), and 6.7% in men specifically accessing VCT from a sample of 2230 persons.

A study in Freetown[12], reported a prevalence of 14.89% HIV infection seen in a private clinic in the year 2000 compared to 9.25% in the year 1999; and in 2006, another study[3] reported a prevalence of 11.4%(937/8,251) after monitoring VCT and women reporting for antenatal care in 7 hospitals in Freetown.

Further, blood chemistry of HIV positive patients were mostly healthy with few values beyond their critical values necessitating the need to improve the diet of the patients.

TABLE 6.1: BLOOD CHEMISTRY ANALYSES OF HIV INFECTED PERSONS

Subject	Parameter	Hb	HCT	Glucose	iCa)	Creatinine	Potassium ions	Chloride ions	carbon dioxide (TC02)	urea nitrogen (BUN)	sodium ion (Na)	anionic gap (AnGap)
Male	Mean±SD	12.82±2.88	37.65±8.57	100.44±25.82	1.11±0.29	1.19±0.42	3.87±1.38	103.94±8.36	22.35±7.3	15.35±13.26	134.65±7.26	15.07±3.1
	Reference Range	12-17	38-51	70-105	1.2-1.32	0.6-1.3	3.5-4.9	98-105	24-29	8-26	138-146	24-29
	Median	12.20		94.5	1.14	1.20	3.5	104	23.0	10	136	14.0
Female	Mean±SD	11.02±3.68	32.73±10.88	112.07±54.30	1.06±0.11	1.38±0.83	4.08±1.04	103.4±3.48	21.5±3.63	11.13±11.6	135.07±	14.21±3.26
	Reference Range	12-17	38-51	70-105	1.2-1.32	0.6-1.3	3.5-4.9	98-105	24-29	8-26	138-146	24-29
	Median	10.90		101.0	1.08	1.0	3.9	104	21.5	6	136	14.0

6.5 CONCLUSION

Few studies have reported the prevalence of HIV in Sierra Leone. This study provides additional evidence that the HIV epidemic is serious in the City of Bo Sierra Leone. Since only one catchment population was assessed for HIV, it is possible that this is just a hotspot, and the result may not apply to the entire city of Bo or Sierra Leone. An independent country-wide prevalence of HIV is recommended.

6.6 REFERENCES

1. UNAIDS and World Health Organization. AIDS epidemic update December 2005. World Health. 2005 p. 1–98.

2. UNAIDS. Global Report: UNAIDS Report on the Global AIDS Epidemic 2013. 2013 p. 198.

3. Ansumana, R; Sundufu A. THE MANAGEMENT OF HIV/AIDS IN FREETOWN, SIERRA LEONE: A STUDY OF HIV/AIDS IN SIERRA LEONE. LAP LAMBERT Academic Publishing; 2010.

4. Secretariat NA. Sierra Leone UNGASS PROGRESS REPORT 2012. Freetown; 2012 p. 26.

5. Chonghaile CN. World Bank allocates funds to help war-torn Sierra Leone fight HIV/AIDS. Lancet [Internet]. 2002 Apr 6 [cited 2014 Oct 6];359(9313):1219. Available from http://www.ncbi.nlm.nih.gov/pubmed/11955553

6. NAS/MOHS. National Population-Based HIV Seroprevalence Survey (2005) Sierra Leone. Freetown; 2005.

7. UNAIDS. Sierra Leone Country Report on Declaration of Commitment on HIV and AIDS (2006 –2007): Prepared for the UN General Assembly on HIV and AIDS(UNGASS). 2008;

8. Bhoobun S, Jetty A, Koroma MA, Kamara MJ, Kabia M, Coulson R, et al. Facilitators and barriers related to voluntary counseling and testing for HIV among young adults in Bo, Sierra Leone. J Community Health [Internet]. 2014 Jun [cited 2014 Oct 7];39(3):514–20. Available from http://www.ncbi.nlm.nih.gov/pubmed/24203408

9. Ansumana R, Jacobsen KH, Gbakima AA, Hodges MH, Lamin JM, Leski TA, et al. Presumptive self-diagnosis of malaria and other febrile illnesses in Sierra Leone. Pan Afr Med J [Internet]. 2013;15:34. Available from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3758859&tool=pmcentrez&rendertype=abstract

10. Bioline S. The 4th Generation of ONE STEP HIV antigen and antibodies to HIV-1/2 Test: SD Bioline HIV Ag/AB Combo. Kyonggi-Do, Korea; 2010.

11. Kouyoumdjian FG, Seisay AL, Kargbo B, Khan SH. The voluntary HIV counselling and testing service in Kenema District, Sierra Leone, 2004-2006: a descriptive study. BMC Int Health Hum Rights. 2010;10:4.

12. Willoughby VR, Sahr F, Russell JB GA. The usefulness of defined clinical features in the diagnosis of HIV/AIDS infection in Sierra Leone. Cell Mol Biol. 2001;47(7):1163–7.

CHAPTER SEVEN

COMMON FEBRILE ILLNESSES IN SIERRA LEONE: ETIOLOGY OF UNDIFFERENTITAED FEBRILE ILLNESSES

7.1 ABSTRACT

Background

Undifferentiated febrile illnesses are common in resource-poor countries that are home to multiple infectious diseases. Bacterial pathogens remain endemic together with presumptive self-diagnosis and self-treatment practices, while patterns of resistance keeps changing with several reports of multi-drug resistance.

Objectives

This study aims at investigating the etiology and prevalence of undifferentiated febrile illnesses in Bo, Sierra Leone.fc

Methods

Blood, urine and stool samples were obtained from febrile patients recruited from the catchment of Mercy Hospital in Bo. A tiered laboratory analyses was done involving LFIs, PCR, Culture and RPM.

Results

Only 2 %(29/1403) were positive for typhoid fever, but seroprevalence of positive widal titres were 57.2% for Salmonella typhi 'O' and 62.3% for Salmonella typhi 'H' at ≥120 titres. Neglected tropical diseases detected included: 19.3% *Ascaris lumbricoides* (95% CI: 14.2-25.8); 10.8% hookworms (95% CI: 7.0-16.3); 6.3% *Schistosoma mansoni* (95% CI: 3.5-10.8); 1.14% *Schistosoma haematobium*; 1.2% Strongyloides *stercoralis* (95% CI: 0.3-4.1); and 2.8% *Trichuris trichiura* (95% CI: 1.2-6.5). Several bacterial pathogens and viruses were also detected.

Conclusion

Bacterial, viral, protozoan and helminthes are among frequent etiologic agents that cause undifferentiated fever in Bo, Sierra Leone. Among the bacteria, Salmonella enterica serotype Typhi is of importance as the population antibody levels has risen such that about three-fifth of the study population had up to 1:120 titers of both Anti-O and Anti-H antibodies. A new cut-off point for the Widal test at 1:180 or above is recommended to prevent over prescription of antibiotics for cases not related to typhoid.

7.2 INTRODUCTION

Fever typically originates from infection [1,2]. It is a prominent clinical indicator of disease processes in a mammalian host.[3] Fever is also an ancient adaptive compensatory defense mechanism that activates the immune system; resulting in a decrease in pathogen growth rate and an increase in host survival, [4]. It is a regulated increase in body temperature above normal and mechanistically distinct from hyperthermia, [5].

Infections resulting to fever can either be acute or chronic. Both acute and chronic undifferentiated febrile illnesses are common in resource-poor countries that are home to multiple infectious diseases. When an acute undifferentiated fever becomes chronic, lasting weeks, it becomes a fever of unknown origin, though the two terms are sometimes used interchangeably.

Bacterial pathogens such as human Salmonella[6,7,8] causing typhoid and paratyphoid fevers, and non-typhoidal salmonellosis remain endemic in many poor countries of the world[9,10] in addition to other bacterial infections such as cholera[11–13] and tuberculosis[14]. The gold standard for diagnosing typhoid fever is by culture[15]. Culture has a sensitivity of 40-60%[16], but low-cost tests for enteric fever, mainly the Widal Test, are more adaptable to resource poverty and are commonly used in resource-poor settings[17]. Widal tests have been in use for over 110 years, but the results are very controversial[17–19] and the test suffer from low specificity in endemic countries due to rise in population antibody levels[18]. Presumptive self-diagnosis and self-treatment, practices in these countries remain common,[20] while patterns of resistance in Salmonella keeps changing with many reports of multi-drug resistant salmonellae[21–23].

Understanding the undifferentiated febrile illnesses of a location requires multiple microbial investigations such as bacteria in the urine, stool or blood samples; investigating other etiologic agents such as helminths, viruses or protozoans. There is also a need for multiple diagnostic approaches. These are difficult tasks in resource-poor settings, where diagnostic

resources are limited, and people have been exposed to several infectious diseases to an extent that they feel experienced in self-diagnosing.

Bacterial infections may be frequent, but knowing the cause of a bacterial infection does not suffice to provide adequate therapeutics; it is also important to understand if available antibiotics are useful, because in resource-poor environments, antimicrobial resistance may occur due to excessive use of over-the-counter drugs without prescription and little studies have been done to account for the extent of such a pattern in Sierra Leone. Among the few studies done include reports by Leski et al[24,25] who reported tet(X) gene[24], that encodes a flavin-dependent monooxygenase that confers resistance to all clinically relevant tetracycline antibiotics including tigecycline and multiple carbapenemase genes[25] from clinical isolates in Bo Sierra Leone.

This study aims at investigating the etiology and prevalence of undifferentiated febrile illnesses in Bo, Sierra Leone.

7.2 METHODS

This laboratory-based study was preceded by a syndromic surveillance survey described elsewhere[20] where 60% of research participants indicated that they self-treat when febrile and indicated several infectious diseases they suspect to have when febrile including typhoid fever, malaria and a host of others.

A cohort of 1403 individuals was followed for a year and their febrile illnesses investigated using a tiered laboratory analyses method comprising several diagnostic tests.

7.2.1 Rapid diagnostic tests

Blood samples collected from febrile subjects were used, with 500µl-1500 µl of blood used for analyses. Up to 500µl -1000 µl was used for storage and/ further immunochromatographic test, (ICT) or lateral flow assay (LFA) analysis as required. ICT tests used about 5 -100µl of blood. The following rapid tests were used: Chikungunya,

malaria, typhoid fever, syphilis, HIV 1 and 2, dengue fever, leptospira, influenza A and B, RSV and Strep A, HBsAg and Hepatitis Combo (HBsAg, HBeAg, HBsAb HBeAb, HBcAb), Hepatitis C Virus, HCV and Hepatitis A Virus, HAV IgG/IgM (Standard Diagnostics, Seoul, Korea). Images of ICT tests were uploaded via a portable, battery-operated LF assay reader/imager (Deki reader, Fio Corporation, Toronto, Canada) to a cloud database at www.fio.net.com for a quality assessment (Fig 4.1). The automated reader has been used variously to confirm rapid lateral assays with congruent results[26,27].

Widal tests were used for detection of antibody levels for Salmonella. Febrile antigens kits (Biorex Diagnostics Ltd, Technology Park, Belfast Road, Antrim) were used to test for Salmonella typhi using qualitative slide agglutination and semi-quantitative tube agglutination tests. Screening for the presence of Salmonella typhi "O' and Salmonella surface "H" antibodies was done with the slide test using blood serum as sample. A drop of each of the "O" and "H" antigens in the Febrile Illness test kit, on blood serum, rotated at 100RPM for a minute. Agglutination is indicative of a recent or current typhoid fever infection. A tube agglutination test was done on reactive slide agglutination samples. Applications of serum samples and antigen drops in varying dilutions such as 1:20, 1:40, 1:80, 1:120, 1:160, 1:200, and 1:240 up to 1:640 constituted the test. An antibody titre of 1:160 done once or higher (without serial testing) was a positive result for Salmonella antibodies in the patient.

7.2.2 Blood chemistry and urinalyses

Additionally, blood chemistry analyses were done using the iStat 300 platform. Moreover, urinalyses were also done using the urine dipstick, Multistix® 10SG (Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA). Centrifugation and microscopic smear were done with the Bayer Atlas of Urine Sediments (Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA).

7.2.3 Tuberculosis tests

The SD Bioline TB antibody tests were done. The Rapid TB Test is an in vitro immunochromatographic test for the detection of antibodies to *Mycobacterium tuberculosis*

in human serum, plasma. The TB point-of-care test is reported to have no cross-reactivity with other species of Mycobacterium and does differential detection of tuberculosis IgG and IgM with a sensitivity of 98.2%.

In addition, Ziehl–Neelsen staining was done to detect TB microscopically. TB samples were obtained three times: spot, early morning and spot. Slides with smear were placed upwards, on the staining rack over a sink, about 1 cm apart. New filter papers were put in a small funnel over the slides and filled with carbol-fuchsin staining solution, so that the solution filters through the paper, covering each slide completely. Slides were heated by a torch prepared by burning cotton wool in ethanol and moved over them perpetually until steam rises; repeating the process twice at intervals of 3-5 minutes. The solution was prevented from drying, and fresh stain was added until staining contact time is at least 10 minutes. Slides were tilted to drain off the stain solution and then rinsed with reverse osmosis (RO) water or distilled water. Acid solution was then added over the smears, covering them completely for 3 minutes. They were drained of the acid and rinsed again. If macroscopically stains were still present, the acid treatment was repeated, followed by methylene blue stain for 1 minute. The slide was rinsed afterwards, dried and ready for use.

7.2.2 Culture and antimicrobial resistance testing

Urine and stool samples were cultured on agar media (Hardy Diagnostics, Santa Maria, CA) or CHROMagar™ plates (CHROMagar, Paris, Fr.).Chromagar is powder based media with agar, peptone and yeast extracts and chromogenic mix for differentiation or group identification. Other types of media used were: Brain Heart Infusion (BHI) Agar, Tryptic Soy Agar (TSA), MacConkey agar, Hektoen enteric (HE) agar, Salmonella Shigella Agar (SS) (Hardy Diagnostics, Santa Maria, CA).

For blood culture, blood samples were collected using aseptic techniques involving alcohol wipes, BD Vacutainer®Safety-Lok™blood collection set with pre-attached holder (Becton Dickinson and Company, Franklin Lakes, NJ, USA). Blood culture was done using the Oxoid SIGNAL (Oxoid Ltd., Basingstoke, United Kingdom) blood culture system. The constitution of the medium encourages the growth of aerobic, anaerobic and microaerophilic organisms. Gas produced by bacterial metabolism, for a positive culture, makes the medium from the

culture bottle to move to an upper reservoir via a hollow needle. Displacement of media may provide a visual indication of the presence of both aerobic and anaerobic organisms in a single medium[28].

API20E for identification was used in concert with other methods of identification such as by the CHROMagar plates, or by PCR. The API-20E test kit (bioMerieux, Inc., Hazelwood, MO), is a standardized identification system for Enterobacteriaceae and other non-fastidious gram-negative rods and it constitutes a plastic strip with twenty-one miniaturized biochemical tests and a database with results. The mini-test tubes (20 in all) were inoculated with a saline suspension of a pure culture thereby providing raw sample and also rehydrating desiccated medium in each tube. The tube and cupules of mini tubes CIT, VP and GEL, were filled completely with culture suspension. While filling only the tube and not the cupule of mini tubes ADH, LDC, ODC, H_2S, and URE and then overlaying with mineral oil to facilitate anaerobic reactions. The strip is incubated in an oven at 37°C for 18-24 hours. During incubation, bacterial metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. After an incubation a TDA Test is done by adding one drop of TDA reagent to the TDA mini-tube: a reddish brown color indicates a positive reaction to being recorded on the provided result sheet. The VP test is also done by adding one drop each of VP1 and VP2 reagents to the VP mini-tube, and a positive outcome (pink or red) obtained after 10 minutes, but slightly pink is negative. An IND test is also done by adding one drop of JAMES reagent to the IND mini-tube; a pink colour is a positive IND test. All positive colour readings add up to number codes, seven digits in all, called the analytical profile index or API that pinpoints the genus and species of bacterium present in the sample (Table 1). The reading was done from an API Reference Book or accessed from the apiweb™ at https://apiweb.biomerieux.com.

Fig. 7.1: API Identification

7.2.3 Sensitivity Tests

Pure isolates were subcultured with sensitivity disks. The single isolated colonies grown on agar medium (Hardy Diagnostics, Santa Maria, CA) were transferred into tubes containing 0.9% sterile saline solution and were emulsified. The inoculum was then used to streak Mueller–Hinton agar plates (Hardy Diagnostics, Santa Maria, CA), and antibiotic-containing disks including azithromycin(15µg), gentamicin(10 µg), kanamycin(30 µg), ampicillin/sulbactam(20 µg), sulfisoxazole (30 µg), ciprofloxacin(5 µg), chloramphenicol(30 µg)and doxycycline(30 µg)((Hardy Diagnostics, Santa Maria, CA), were dispensed onto the surface. Antibiograms were generated on each isolate using standard disk diffusion methods specified by the Clinical and Laboratory Standards Institute (CLSI) guidelines. All plates incubated for18-24 hours at 37˚C, and the diameters of inhibition zones were then measured and interpreted.

7.2.4 Sequencing and multiplex reactions

Single colony isolates were obtained using flamed loops and transferred to 1.5ml Eppendorf tubes for total genomic DNA extraction using a MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI). The isolates were identified genetically after PCR amplification and sequencing of a 16S rDNA amplicon that spanned the V3 and V4[25] variable regions and the rpoB or gyrA gene previously described[29,30,24].

Blood culture results were obtained by using the FilmArray multiplex PCR platform (FA; BioFire, Salt Lake City, UT)) using the Blood culture Identification (BCID) Pouch. The methods of the test have been previously described [31].

The Biothreat panel of the Filmarray system described previously[32] was also used together with the respiratory pouch. Pathogens that can be detected by the blood culture identification panel (BCID) and the Biothreat Panel (BT Panel) are indicated in the following tables:

Table 7.1: Filmarray bcid panel

Gram Positive Bacteria	Klebsiella oxytoca	Candida krusei
Enterococcus species	Klebsiella pneumonia	Candida parapsilosis
Listeria monocytogenes	Serratia marcescens	Candida tropicalis
Staphylococcus aureus	Proteus Haemophilus influenzae	
Streptococcus species	Neisseria meningitidis	**Antibiotic resistance genes**
Streptococcus agalactiae	Pseudomonas aeruginosa	mecA gene
Streptococcus pneumoniae	**Fungi**	KPC gene
Streptococcus pyogenes	Candida albicans	vanA gene
Gram Negative Bacteria	Candida glabrata	vanB gene
Enterobacter cloacae		
Escherichia coli		

Table 7.2: FilmArray Biothreat Panel

Bacillus anthracis	*Ricinus communis*	Orthopox genus virus
Brucella melitensis	*Rickettsia prowazekii*	Variola virus
Burkholderia mallei/pseudomallei	*Staphylococcal enterotoxin gene*	EEE virus
Clostridium botulinum	*Yersinia pestis*	VEE virus
Coxiella burnetii	Ebola Zaire	WEE virus
Francisella tularensis	Marburg virus	

7.3 RESULTS

7.3.1 Enteric Fever

Of the 1403 febrile subjects enrolled in this study, 1095 were screened by the Widal test that was used to screen for antibody levels to Salmonella typhi in the population. 57.2%(626/1095) were having ≥120 titres of Salmonella typhi "O" ,62.3% (682/1095) were having ≥120 titres of Salmonella typhi "H" antibodies; this represents the reported rate of typhoid fever locally based on titers of 1:120 which is used in many clinics in Sierra Leone as a positive result. 109(10%) had 140 titres of Salmonella "O" and 101(9.2%) had 140 titers of Salmonella "H" antibodies. The remaining, 360(32.9%) and 312(28.5%) were having ≥160 titers of Salmonella typhi "O" and "H' antibodies respectively. Titre values of ≥160 done once were considered presumptively positive for typhoid fever infection.

However, further microbiological testing yielded only 2% (29/1403) positive cases of typhoid fever (Table 7.3).

7.3.2 Helminthiasis

Ova of several helminths were detected in stool samples of subjects and considered important in the non-malarial febrile ecology in Bo, Sierra Leone. Out of 176 stool samples examined for worms (Table 7.3), 19.3% had *Ascaris lumbricoides* (95% CI: 14.2-25.8); 10.8% had hookworms (95% CI: 7.0-16.3); 6.3% had *Schistosoma mansoni* (95% CI: 3.5-10.8); 1.14% had *Schistosoma haematobium*; 1.2% Strongyloides *stercoralis* (95% CI: 0.3-4.1); and 2.8% had *Trichuris trichiura* (95% CI: 1.2-6.5).

Schistosoma spp. *Trichuris trichiura* are neglected tropical diseases and also known as diseases of poverty.

7.3.3 Non-typhoidal Bacterial infections

Several bacterial pathogens were detected in samples that were analyzed by culture from stool, urine and blood and sputum smear. Frequent bacterial infections included: 16.9% of *Escherichia. coli* (95%CI:11.6-23.9); 12.6% of *Klebsiella pneumonia* (95%CI: 8.2-19.2); 12% of *Citrobacter freundii (*95%CI:7.6-18.3); 8.5% of *Enterobacter cloacae (95%CI:* 4.9-14.2), 7.5% *Haemophilus influenzae* (95%CI: 3.7-14.2), *5%Chlamydophila pneumonia (95%CI:* 2.9-11.6), 3.3% *Moraxella catharrhalis* (95%CI: 1.3-8.3), 2.8% *Kluyvera spp. And 2.8% Serratia plymuthica /marcescens* (95%CI: 1.1-7.0), 2.5% *Mycoplasma pneumonia* (95%CI: 0.9-7.1), 1.6% *Treponema pallidum* (95%CI: 1.1-2.5*)* and 0.7% *Enterobacter intermedium, 0.7 Enterobacter aerogenes and 0.7% Escherichia hermannii (95%CI:* 0.1-3.9). *(Table 7.3).*

7.3.4 Viral Infections

Viral infections included 46% Chikungunya (95%CI 43.5-48.7), 24.2 Human Rhino virus/enterovirus (95%CI: 17.4-32.6), 19.2% Corona virus (95%CI: 13.1-27.1), 9.7% HIV (95%CI: 8.2-11.4), 8.5% Hepatitis B(HbSAg) (95%CI 7.1-10.1), 8.7%HAV(IgG)(95%CI: 7.3-10.3), 8.3% Influenza B(95%CI: 4.6-14.7), 5% Adenovirus(95%CI: 2.1-11.0), 4.7% Hepatitis C(95%CI: 3.7-5.9), 2.8% dengue fever (95%CI: 2.0-3.8), 1.7% parainfluenza virus and 1.7% Influenza A(H1N1) (95%CI: 0.5-5.9), 0.8% cytomegalovirus(95%CI: 0.04-5.2) and 0.2 % Human Coxsackie virus A24 and A22(95%CI: 0.07-0.6) were detected (Table 7.3).

Table 7.3: Fever Causing Pathogens Identified by a Tiered Laboratory Analyses (LFI's, Microscopic Smear, API20E & 32E, Multiplex PCR, Rpob Sequencing and 16s sequencing).

Etiologic Agent Infectious Agent	Type	No. Positive	No. Tested	%Positive of Tested	95%CI
Citrobacter freundii	Bacteria	17	142	12.0	7.6-18.3
Enterobacter cloacae	Bacteria	12	142	8.5	4.9-14.2
Escherichia coli	Bacteria	24	142	16.9	11.6-23.9
Escherichia hermannii	Bacteria	1	142	0.7	0.1-3.9
Klebsiella pneumoniae	Bacteria	19	158	12.0	7.8-18.0
Kluyvera	Bacteria	4	142	2.8	1.1-7.0
Ent. aerogenes	Bacteria	1	142	0.7	0.1-3.9
Ent. Intermedium	Bacteria	1	142	0.7	0.1-3.9
Serratia plymuthica /marcescens	Bacteria	4	142	2.8	1.1-7.0
Mycoplasma pneumonia	Bacteria	3	120	2.5	0.9-7.1
Chlamydophila pneumonia	Bacteria	7	120	5.8	2.9-11.6
Moraxella catharrhalis	Bacteria	4	120	3.3	1.3-8.3
Streptococcus pneumonia	Bacteria	12	120	10.0	5.8-16.7
Haemophilus influenzae	Bacteria	9	120	7.5	3.7-14.2
Treponema palidum	bacteria	23	1403	1.6	1.1-2.5
Salmonella typhi	bacteria	29	1403	2.1	1.5-3.0
Ova of Ascaris lumbricoides	helminth	34	176	19.3	14.2-25.8
Ova of Hookworm	helminth	19	176	10.8	7.0-16.3
Schistosoma masonia	helminth	11	176	6.3	3.5-10.8
Schistosoma haematobium	helminth	2	176	1.1	0.3-4.2
Trichuris trichiura	helminth	5	176	2.8	1.2-6.5
Strongyloides	helminth	1	176	1.1	0.03-3.6
Mycobacterium tuberculosis	bacterium	34	1403	2.4	1.7-3.4
Yersinia pestis	bacterium	16	1403	1.1	0.7-1.8
Burkholderia pseudomallei	bacterium	66	1403	4.7	3.7-5.9
Human coxackie virus A24 and A22.	Viral	3	1403	0.2	0.07-0.6
Chikungunya	viral	646	1403	46.0	43.5-48.7
HIV	Viral	136	1403	9.7	8.2-11.4
HCV	Viral	66	1403	4.7	3.7-5.9
HBsAg	Viral	119	1403	8.5	7.1-10.1
HAV IgG	Viral	122	1403	8.7	7.3-10.3
Dengue	Viral	39	1403	2.8	2.0-3.8
Influenza A	Viral	2	120	1.7	0.5-5.9
Influenza B	Viral	10	120	8.3	4.6-14.7
Cytomegalovirus	Viral	1	120	0.8	0.04-5.2
Corona Virus(0C43)	Viral	23	120	19.2	13.1-27.1
Human Rhino virus/enterovirus	Viral	29	120	24.2	17.4-32.6
Adenovirus	Viral	6	120	5.0	2.1-11.0
Parainfluenza	Viral	2	120	1.7	0.5-5.9
Malaria	protozoan	331	1403	23.6	21.4-25.9

7.3.5 Antimicrobial Resistance

Based on disk diffusion using the CLSI guidelines, the following pattern of resistance was observed in 56 isolates: 85.71% Sulfisoxazole(G), 82.14% gentamycin(GM), 78.57% Chloramphenicol(C), 58.9% aztreonam(ATM) 53.6% Ciprofloxacin(CIP), 51.8% ceftazidime(CAZ), 42.86% doxycycline(D) and 32.1% azithromycin(AZM)(Table 7.4). Tigecycliness (TGC) and impinem (IMP) were the most susceptible antibiotics.

Table 7.4: Antibiograms Generated from Clinical Isolates

Antimicrobial Agents	**AZM**	**ATM**	**CAZ**	**CIP**	**IMP**	**TGC**	**C**	**D**	**G**	**GM**	**K**	**SAM**
Total	*56*	*56*	*56*	*56*	*56*	*56*	*56*	*56*	*56*	*56*	*56*	*56*
Mean disk Diffusion (mm)	*16.5*	*17.9*	*18.2*	*14.2*	*24.0*	*19.8*	*10.0*	*11.4*	*6.3*	*10.6*	*16.1*	*11.8*
±SD	*6.1*	*6.1*	*4.9*	*7.2*	*3.3*	*2.2*	*7.32*	*3.72*	*2.41*	*5.99*	*4.0*	*3.58*
Resistance (%)	*32.1*	*58.9*	*51.8*	*53.6*	*7.1*	*0.0*	*78.6*	*42.9*	85.7	*82.1*	*28.6*	*57.1*
Intermediate (%)	*16.1*	*16.1*	*21.4*	*26.8*	*28.6*	*30.4*	*1.79*	*26.79*	*0.0*	*1.79*	*44.64*	*32.1*
Susceptible (%)	*51.8*	*25.0*	*26.8*	*19.6*	*64.3*	*69.6*	*19.64*	*30.36*	*1.79*	*16.07*	*26.79*	*10.7*

AZM:azithromycin, ATM:aztreonam, CAZ:ceftazidime, CIP: Ciprofloxacin, TGC:Tigecycline, IMP:impinem, C:chloramphenicol, D:Doxycycline, G:Sulfisoxazole, GM:Gentamycin, K:Kanamycin, SAM: ampicillin/Sulbactam.

Molecularly, the following resistant genes were detected: quinolone resistant gene (QNR), C2, tet-X; blaOXA-51-like, blaOXA-58, blaDIM-1 and blaVIM carbapenemase genes.

7.4 DISCUSSION

The study population has high antibody levels for Salmonella enterica serotype Typhi with almost 60% having ≥120 titers of anti-O and anti-H antibodies. High antibody levels are associated with less reliability for the Widal test[18] especially when the levels do not

correspond to concomitant microbial detections by other assays such as culture. The high antibody levels in this study do not correspond with the detections of Salmonella enterica serotype Typhi pathogens and could be due to cross-reacting antigens. There exist up to 40 cross-reacting antigens between Salmonella enterica serotype Typhi and other enterobacteriaceae[33], in addition, cross-reacting antigens could also be from malaria, brucellosis, dengue fever, chronic liver disease or endocarditis[34].

The result also shows that multiple etiologic agents are responsible for undifferentiated fever in Bo, Sierra Leone. The bacterial pathogens detected in this study probably constitute a fraction of all bacteria that routinely infect people in Bo Sierra Leone. Since presumptive treatment is common in this location[20] both at the healthcare centers and at home to deal with undifferentiated fevers, there is a possibility that even though pathogens are unknown they could be treated by the use of broad-spectrum antibiotics such as ciprofloxacin in the study location. However, the existence of resistance to ciprofloxacin (53.6%) which is usually a drug of choice is a grave concern. Also the observed resistance and resistant genes such as QNR, C2, tet-X; blaOXA-51-like, blaOXA-58, blaDIM-1 and blaVIM carbapenemase genes, mean that not all infections can be treated timely without combination therapy with multiple antibiotics.

Further, the detections of pathogens *Schistosoma spp*. *Trichuris trichiura and Wuchereria bancrofti* which cause neglected tropical diseases is of importance, as over three rounds of mass drug administration consisting a combination of Albendazole and Ivermectin have been done in Sierra Leone to eliminate the transmission of helminths associated with NTDs[35,36]. Thus, more MDAs could benefit the population to reduce transmission potential of NTDs further.

And the detection of *Yersinia pestis* is an importrant public health finding, as it causes plague. Plague, a murine zoonosis, is caused by *Yersinia pestis*, a gram-negative, non-motile, non-sporulating, rod-shaped, bacterium of the Enterobacteriaceae family. The disease affects humans, when they contact infected rodents, their respiratory secretions, or are bitten by fleas. Humans can also get it through inhalation of aerosolized droplets from infected persons, consumption of contaminated food or by laboratory exposure[37–40].

Clinically, plague occurs in three forms: bubonic plague, septicemic plague and pneumonic plague[41]. Bubonic plague is characterized by fever, chills, weakness, headache and swellings of lymph nodes or buboes commonly in the inguinal and groin femoral regions [39,41]. Septicemic plague normally progresses from bubonic plague, but lacks buboes and characterized by fever, gastrointestinal symptoms, such as nausea, vomiting, diarrhea and abdominal pain; hypotension, disseminated intravascular coagulation, and multiorgan failure develop in the later stages of the illness[42]. Pneumonic plague is characterized by sudden onset of dyspnea, high fever, pleuritic chest pain, and cough that may be accompanied by characteristic bloody sputum. Primary pneumonic plague is rapidly fatal unless an appropriate antimicrobial agent is begun within the first day of illness[42].

Similarly, *Burkholderia pseudomallei*, which was detected is believed to cause melioidosis which kills between 20-50% of positive cases.[43] Melioidosis is rare in Africa, but was reported in a Sierra Leonean based in Gambia in 1985[44], the disease is common in Southeast Asia and northern Australia[45].

7.5 CONCLUSIONS

Bacterial, viral, protozoan and helminthes are among frequent etiologic agents that cause undifferentiated fever in Bo, Sierra Leone. Among the bacteria, Salmonella enterica serotype Typhi is of importance as the population antibody levels has risen such that about three-fifth of the study population had up to 1:120 titers of both Anti-O and Anti-H antibodies. A new cut-off point for the Widal Test above 1:120 is recommended to prevent over prescription of antibiotics for cases not related to typhoid, however the new cut-off point should be determined by additional studies to determine the sensitivity and specificity of widal at different titre values.

Combination therapy with antibiotics should be emphasized, and the inclusion of tigecycline and imipenem in the combination therapy should be encouraged since they have lower resistance to them from bacterial pathogens in this study. However, the existence of QNR genes and carbapenemase resistance genes suggests that more resistance should be expected

in the near future, something that can be averted by discouraging over-the-counter purchase or prescription drugs without medical advice.

7.6 REFERENCES

1. Bartfai T, Conti B. Fever. ScientificWorldJournal 2010;10:490–503.

2. Mackowiak PA. Fever: blessing or curse? A unifying hypothesis. Ann Intern Med 1994;120:1037–40.

3. Ogoina D. Fever, fever patterns and diseases called “fever” - A review. J. Infect. Public Health. 2011;4:108–24.

4. Mackowiak PA. What we do when we suppress fever. Curr. Infect. Dis. Rep. 2005;7:1–4.

5. Cannon JG. Perspective on fever: The basic science and conventional medicine. Complement. Ther. Med. 2013;21.

6. Mamo Y, Belachew T, Abebe W, Gebre-Selassie S, Jira C. Pattern of widal agglutination reaction in apparently healthy population of Jimma town, southwest Ethiopia. Ethiop Med J 2007;45:69–77.

7. Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. Invasive non-typhoidal salmonella disease: An emerging and neglected tropical disease in Africa. Lancet. 2012;379:2489–99.

8. Leski TA, Ansumana R, Malanoski AP, et al. Leapfrog diagnostics: Demonstration of a broad spectrum pathogen identification platform in a resource-limited setting. Heal. Res. Policy Syst. 2012;10:22.

9. Parry CM, Wijedoru L, Arjyal A, Baker S. The utility of diagnostic tests for enteric fever in endemic locations. Expert Rev Anti Infect Ther 2011;9:711–25.

10. Kingsley RA, Msefula CL, Thomson NR, et al. Epidemic multiple drug resistant Salmonella Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. Genome Res 2009;19:2279–87.

11. Von Nguyen D, Sreenivasan N, Lam E, et al. Cholera epidemic associated with consumption of unsafe drinking water and street-vended water-Eastern Freetown, Sierra Leone, 2012. Am J Trop Med Hyg 2014;90:518–23.

12. Dyer O. Cholera epidemic threatens Sierra Leone. BMJ. 1995;311:77.

13. WHO. Cholera in Sierra Leone - update [Internet]. Glob. Alert Response. 2012; Available from: http://www.who.int/csr/don/2012_09_18b/en/index.html

14. Homolka S, Post E, Oberhauser B, et al. High genetic diversity among Mycobacterium tuberculosis complex strains from Sierra Leone. BMC Microbiol 2008;8:103.

15. Prakash P, Mishra OP, Singh AK, Gulati AK, Nath G. Evaluation of nested PCR in diagnosis of typhoid fever. J Clin Microbiol 2005;43(1):431–2.

16. WHO. Background document: the diagnosis, treatment and prevention of typhoid fever.Vaccines and Biologicals. 2002.

17. Olopoenia LA, King AL. Widal agglutination test - 100 years later: still plagued by controversy. Postgrad Med J 2000;76:80–4.

18. Clegg A, Passey M, Omena M, Karigifa K, Suve N. Re-evaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in the highlands of Papua New Guinea. Acta Trop 1994;57:255–63.

19. Onuigbo MAC. Diagnosis of typhoid fever in Nigeria: Misuse of the Widal test. Trans R Soc Trop Med Hyg 1990;84:129–31.

20. Ansumana R, Jacobsen KH, Gbakima AA, et al. Presumptive self-diagnosis of malaria and other febrile illnesses in Sierra Leone. Pan Afr Med J [Internet] 2013;15:34.

Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3758859&tool=pmcentrez&rendertype=abstract

21. Kariuki S, Revathi G, Kariuki N, et al. Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994-2003. Int J Antimicrob Agents 2005;25:38–43.

22. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. Curr Opin Infect Dis 2008;21:531–8.

23. Kaur J. Increasing antimicrobial resistance and narrowing therapeutics in typhoidal Salmonellae. J. Clin. Diagnostic Res. 2013;7:576–9.

24. Leski TA, Bangura U, Jimmy DH, et al. Multidrug-resistant tet(X)-containing hospital isolates in Sierra Leone. Int J Antimicrob Agents 2013;42:83–6.

25. Leski T a, Bangura U, Jimmy DH, et al. Identification of blaOXA-51-like, blaOXA-58, blaDIM-1 and blaVIM Carbapenemase Genes in Hospital Enterobacteriaceae Isolates from Sierra Leone. J Clin Microbiol [Internet] 2013;51:2435–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23658259

26. Herrera S, Vallejo AF, Quintero JP, Arévalo-Herrera M, Cancino M, Ferro S. Field evaluation of an automated RDT reader and data management device for Plasmodium falciparum/Plasmodium vivax malaria in endemic areas of Colombia. Malar J [Internet] 2014 [cited 2014 Sep 25];13:87. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3995821&tool=pmcentrez&rendertype=abstract

27. Shekalaghe S, Cancino M, Mavere C, et al. Clinical performance of an automated reader in interpreting malaria rapid diagnostic tests in Tanzania. Malar J [Internet] 2013;12:141. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23617722\nhttp://www.biomedcentral.com/content/pdf/1475-2875-12-141.pdf

28. Trombley C, Anderson JD. SIGNAL blood culture system for detection of bacteremia in neonates. J Clin Microbiol 1987;25:2098–101.

29. Tayeb LA, Lefevre M, Passet V, Diancourt L, Brisse S, Grimont PAD. Comparative phylogenies of Burkholderia, Ralstonia, Comamonas, Brevundimonas and related organisms derived from rpoB, gyrB and rrs gene sequences. Res Microbiol 2008;159:169–77.

30. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 2007;73:5261–7.

31. Altun O, Almuhayawi M, Ullberg M, Ozenci V. Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol [Internet] 2013;51:4130–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24088863

32. Seiner DR, Colburn HA, Baird C, et al. Evaluation of the FilmArray® system for detection of Bacillus anthracis, Francisella tularensis and Yersinia pestis. J Appl Microbiol [Internet] 2013 [cited 2014 Nov 24];114(4):992–1000. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3617465&tool=pmcentrez&rendertype=abstract

33. Parry CM, Hoa NT, Diep TS, Wain J, Chinh NT, Vinh H HT, White NJ FJ. Value of a single-tube widal test in the diagnosis of typhoid fever in Vietnam. J Clin Microbiol 1999;37:2882–6.

34. Colle JG, Frason AG, Marmion BP SAM and MC. Practical Medical Microboiology. New York: Churchill Livingston; 1996.

35. Hodges MH, Smith SJ, Fussum D, et al. High coverage of mass drug administration for lymphatic filariasis in rural and non-rural settings in the Western Area, Sierra Leone. Parasit Vectors [Internet] 2010 [cited 2014 Dec 3];3:120. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3018440&tool=pmcentrez&rendertype=abstract

36. Koroma JB, Sesay S, Sonnie M, et al. Impact of three rounds of mass drug administration on lymphatic filariasis in areas previously treated for onchocerciasis in Sierra Leone. PLoS Negl Trop Dis [Internet] 2013 [cited 2014 Dec 3];7(6):e2273. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3681681&tool=pmcentrez&rendertype=abstract

37. Prentice MB, Rahalison L. Plague. Lancet. 2007;369:1196–207.

38. Rollins SE, Rollins SM, Ryan ET. Yersinia pestis and the plague. Am. J. Clin. Pathol. 2003;119 Suppl.

39. Butler T. Plague into the 21st century. Clin Infect Dis 2009;49:736–42.

40. Raoult D, Mouffok N, Bitam I, Piarroux R, Drancourt M. Plague: History and contemporary analysis. J Infect 2013;66:18–26.

41. Rollins SE, Rollins SM, Ryan ET. Yersinia pestis and the plague. Am J Clin Pathol 2003;119 Suppl:S78–85.

42. USAMRIID. Medical Management of Biological Casualties Handbook. 7th ed. Fort Detrick, Maryland: 2011.

43. Wuthiekanun V, Peacock SJ. Management of melioidosis. Expert Rev Anti Infect Ther 2006;4(3):445–55.

44. Birnie E, Wiersinga WJ, Limmathurotsakul D, Grobusch MP. Melioidosis in Africa: should we be looking more closely? Future Microbiol [Internet] 2015;10(2):273–81. Available from: http://www.futuremedicine.com/doi/abs/10.2217/fmb.14.113

45. CDC. Melioidosis [Internet]. [cited 2015 May 31];Available from: http://www.cdc.gov/melioidosis/

CHAPTER EIGHT

8.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This section summarises and concludes the findings of the study. The study looked at a tiered laboratory analyses of common infections to characterise febrile morbidity not related to malaria in Sierra Leone. The results have been presented in six preceding chapters encompassing different non-malarial febrile morbidity in Sierra Leone. An overall summary and conclusion is outlined in the next section, followed by strengths and limitations of the study and recommendations based on study results.

8.1 SUMMARY AND CONCLUSIONS

There was an average monthly, febrile illness of 160.25 SD±10.63 for the twelve months of cohort study, with a significant difference in monthly febrile cases ($P<0.001$). The ages 5 to 14 years had an average of 25.83±7.85 cases per month. Those within 15 to 29 years had average monthly cases of 48.83±6.7. Ages 30 to 44 had 41.66±4.2 while the age group ≥45 years had monthly cases of 43.91±6.7.

Results of this study showed that malaria accounts for less than 50% of febrile illnesses investigated in the study location, but about 60% of participants in the study self-diagnosed and self-treated malaria a month prior to the study. The high rate of self-diagnoses is of concern, since treatment decisions that follow could be wrong, delaying recovery, increasing disease burden with a possibility of nurturing antimicrobial resistance. Among the medications used were herbs with unspecified dosages[1]. While herbs could treat certain diseases, they also have the propensity to delay treatment outcomes or cause severe complications from the toxicity of the herbs.

Common non-malaria febrile illnesses in Bo, Sierra Leone include: Chikungunya which accounts for over 40% of febrile disease morbidity in Bo but exists mainly in a transient form with symptoms such as arthralgia, chills, headaches and back pains, with the latter persisting in affected patients[2]. Having an incidence or person-time rate of 573.7 per 1000 people per year at a 95% confidence interval, the incidence of Chikungunya is higher than that of malaria in this malaria endemic region. In a related study in Kenema which is 40km away from Bo Town, Boissen et al[3] reported 35%(27/77) prevalence of Chikungunya in suspected Lassa Fever cases, indicating that ChikV is not only in Bo, but elsewhere in Sierra Leone.

Another common non-malaria febrile morbidity is viral hepatitis which was detected in this study. Per month, an average of 10.4±1.9SD Hepatitis B and 5.5±2.5SD Hepatitis C were recorded in Bo, Sierra Leone. With about 10% prevalence of hepatitis B and 5% prevalence of hepatitis C. The viral hepatitis morbidity seems grim as treatment options are limited within Sierra Leone. Available drugs are either expensive or hard to get. There is about 9% IgG seroprevalence of viral hepatitis A, but this study did not detect any current infection of HAV. It is possible that sampling at a different time could yield a high prevalence.

Furthermore, this study revealed a high seroprevalence of *Salmonella typhi,* about 60% having ≥ 1:120 titres of typhi 'O' or 'H'. However actual incidence of typhoid infection, diagnosed by microbiological testing was lower at about 2% per year. The disparity between the Widal test and the culture and API identification methods indicate that, the antibody levels of Salmonella has increased in the Bo community. The increase may have resulted from previous infections of S*almonella typhi*. Not all widal positive cases are actually having typhoid fever. This necessitates the revision of the widal titre levels accepted as positive reading, since widal tests are predominantly used in Sierra Leone to test for typhoid fever. To determine a new widal cut-off point, sensitivity and specificity of widal tests at different titre levels should be determined.

HIV, which is still dreaded, is an important part of the non-malaria febrile morbidity in Bo, Sierra Leone. The prevalence of HIV was about 9%; three-fifth of the cases were females and overall about 11% were co-infected with Hepatitis B, while 3 % were having syphilis. HIV patients assessed had a median Hb value of 10.90g/dl and 18.75% of critically low hematocrit values (≤27%PCV). The high prevalence of HIV in Bo is consistent with previous

reports from the city. Kellie(2007)[unpublished dissertation][4] reported a prevalence of 8% and other independent studies have been within the same range[5,6].

Several other infections were also detected including Human Rhinoviruses(24%) corona viruses(19%), E. coli 17%, *Ascaris lumbricoides* infections(19%), *Citrobacter freundii* (12%), *Klebsiella pneumonia* (12%), 11% hookworm infections, 10% *Streptococcus pneumonia,* 9% *Enterobacter cloacae,* 8% *Haemophilus influenza,* 8% Influenza B, 6% *Schistosoma mansonia,* 6% *Chlamydophila pneumonia* and other infections. Worthy of mention among the other infections are 16 cases of *Yersinia pestis* which causes bubonic plague and 66 cases of *Burkholderia pseudomallei* which causes melioidosis. Melioidosis is rare in Africa, but was reported in a Sierra Leonean based in Gambia in 1985[7]. Thus Bo city is home to diversity of infections.

Syndromic definitions of febrile illnesses in this location cannot be used as a proxy of actual laboratory diagnosis because syndromes are not specific. Treatments based solely on syndromes is therefore presumptive or assumptive. To avoid assumptive treatments, point of care or point-of-need testing need to be adopted for malaria, where febrile subjects are first tested in their remote locations by themselves or by community volunteers within their communities.

Roukens and colleagues, demonstrated the possibility of malaria-self test and reported 3 %(18/575) invalid results from self-test run by oil expatriates and compliance was higher with instructions.

In Bo, Sierra Leone, Ranasinghe and colleagues[1] reported preferences of malaria testing in Bo with 69% rural residents, preferred a self/family- or CHV-conducted home-based malaria test and 20% a laboratory-based test while urban residents preferred 38% and 44%, respectively. If offered a home-based test, 28% of rural residents would prefer a self/family-conducted test and 68% would prefer a CHV-assisted test[1].

8.2 STRENGTHS AND LIMITATIONS OF THE STUDY

This study has a key limitation of been too broad. Consequently, the scope of the investigations of individual pathogens was limited. Some pathogens have unique methods for investigating them, rather than the methods designed by this study, so it was impossible to deeply carry out analyses of each pathogen investigated at an individual level.

Secondarily cost limitations prevented carrying out initial baseline screening of all subjects thereby limiting the ability to determine incidence of diseases that exist as chronic infections even though tests targeted febrile subjects within 72 hours of becoming febrile.
Furthermore, usage of subjects to self-diagnosis and presumptively treat themselves affected effectiveness of the crowdsourcing system, such that subjects reported fever when the felt they needed to rather than on onset.

Despite having good infrastructure for sample analyses, the lab was under biosafety level 3 and therefore unfit to probe further into certain cases such as Lassa, and Ebola and *Yersinia pestis.* So the one Lassa case that was detected was sent to the Kenema Lassa Lab for testing. *Yersinia pestis* which can best be confirmed by microbial culture and isolation of the bacterium was only confirmed molecularly for fear of aerosolizing the pathogen from cultures.

On the other hand, this study has many firsts in Sierra Leone. It is the first to report the re-emergence of Chikungunya in Sierra Leone[8]. Chikungunya has certain symptoms that persons with malaria could have. Symptoms such as fever, arthralgia and weakness are presumptively associated with malaria within the study community. It was surprising to have a disease with higher prevalence than malaria with the listed symptoms in the study community.

This study also detected 16 *Yersinia pestis* Fraction-1(F1) antigens in febrile subjects in Bo, Sierra Leone and 66 manno-heptose capsular polysaccharide (CPS) antigens for *Burkholderia pseudomallei* which are the causative agents of bubonic plague and melioidoses respectively. F1 is a capsule-like antigen encoded by the *caf1* gene located on the large 100-kb pFra plasmid, which is unique to *Y. pestis*[9]. The positive samples were clinically consistent with YP infection and a positive PCR confirmation was obtained by the

multiplex PCR on BT Panel of the FilmArray Platform (Biofire Diagnostics, Salt Lakes City, US). Even though there was no outbreak of bubonic plague, such a finding is of importance to the public health of Bo, Sierra Leone. Similarly, *Burkholderia pseudomallei* is believed to cause melioidiosis which kills between 20-50% of positive cases[10] but is common in Southeast Asia and northern Australia[11]. Both infections are zoonotic infections, even though Burkholderia could also be obtained from the environment, demonstrating clearly that the closed proximity between animals and humans in Bo, Sierra Leone, coupled with poor environmental sanitation, facilitates ease of transmission of infectious diseases.

Additionally, the resources used in this study became useful to protect healthcare workers during the Ebola outbreak in Sierra Leone. In the study hospital, healthcare workers were routinely screened for Ebola using the BT Panel of the FilmArray, whenever they became febrile and one case who became positive treating a relative, was detected before he became symptomatic, thereby saving lives including mine[12]. The scope of the study also made it possible to contribute to science from a position of strength as demonstrated in the correspondences on point of care tests in the Lancet Infectious Diseases[13], Ebola in the Lancet[14] and New England Journal of Medicine[15]

8.3 RECOMMENDATIONS

It is recommended that point of care tests be made accessible in remote locations to facilitate ease of testing and dissuade from presumptive diagnosis. Since clinicians could easily make misdiagnosis because of overlapping symptoms of diseases in this location, it becomes much more difficult for a non-clinician to make accurate presumptive diagnosis, but POCT can at least exclude diseases such as malaria and also inspire laboratory testing.

Regulating purchase of drugs is highly recommended. Drugs should not be bought without a prescription form. Pharmacies that sell drugs without prescription should be fined or closed. Government should stock cost-recovery drugstores with more drugs to encourage more people to buy there, since they sell based on prescription only.

Widal test should be urgently revised based on evidence from sensitivity and specificity tests done at different titre les Bureau. This is because the population antibody level has risen for Salmonella typhi ‘O’ and ‘H’ making the widal test less specific and seemingly dangerous as it facilitates prescription of antibiotics for typhoid fever, which, if done wrongfully, may increase the tendency for antimicrobial resistance, which is a developing crisis in Sierra Leone.

8.4 REFERENCES

1. Ranasinghe S, Ansumana R, Lamin JM, et al. Herbs and herbal combinations used to treat suspected malaria in Bo, Sierra Leone. J Ethnopharmacol 2015;166:200–4.

2. Roth PJ, Grant DS, Ngegbai AS, Schieffelin J, McClelland RS, Jarrett OD. Factors Associated with Mortality in Febrile Patients in a Government Referral Hospital in the Kenema District of Sierra Leone. Am J Trop Med Hyg [Internet] 2014;92(1):172–7. Available from: http://www.ajtmh.org/cgi/doi/10.4269/ajtmh.14-0418

3. Boisen ML, Schieffelin JS, Goba A, et al. Multiple Circulating Infections Can Mimic the Early Stages of Viral Hemorrhagic Fevers and Possible Human Exposure to Filoviruses in Sierra Leone Prior to the 2014 Outbreak. Viral Immunol [Internet] 2015;28(1):19–31. Available from: http://online.liebertpub.com/doi/abs/10.1089/vim.2014.0108

4. Kelly SJ. Prevalence of HIV in Bo, Sierra Leone. 2007;

5. Kouyoumdjian Dr. F, Seisay AL, Kargbo B, Khan SH. Population seeking voluntary HIV counselling and testing in Kenema district, Sierra Leone. XVII Int. AIDS Conf. 3-8 August 2008, Mex. City, Mex. 2008;

6. Kouyoumdjian FG, Seisay AL, Kargbo B, Khan SH. The voluntary HIV counselling and testing service in Kenema District, Sierra Leone, 2004-2006: a descriptive study. BMC Int Health Hum Rights 2010;10:4.

7. Birnie E, Wiersinga WJ, Limmathurotsakul D, Grobusch MP. Melioidosis in Africa: should we be looking more closely? Future Microbiol [Internet] 2015;10(2):273–81. Available from: http://www.futuremedicine.com/doi/abs/10.2217/fmb.14.113

8. PromedMail. Chikungunya (08): Sierra Leone, RFI [Internet]. 20120720.1209257. 2012 [cited 2015 May 1];Available from: www,promedmail.org/direct.php?id=1209257

9. Du Y, Rosqvist R, Forsberg Å. Role of fraction 1 antigen of Yersinia pestis in inhibition of phagocytosis. Infect Immun 2002;70(3):1453–60.

10. Wuthiekanun V, Peacock SJ. Management of melioidosis. Expert Rev Anti Infect Ther 2006;4(3):445–55.

11. CDC. Melioidosis [Internet]. [cited 2015 May 31];Available from: http://www.cdc.gov/melioidosis/

12. Leski TA, Ansumana R, Taitt CR, Lamin JM, Bangura U, Lahai J, Mbayo G, Kanneh MB, Bawo B, Bockarie AS, Scullion M, Phillips CL, Horner CP, Jacobsen KH SD. Use of FilmArray™ system for detection of Zaire ebolavirus in a small hospital, Bo, Sierra Leone. JCM 2015;53(7):2368–70.

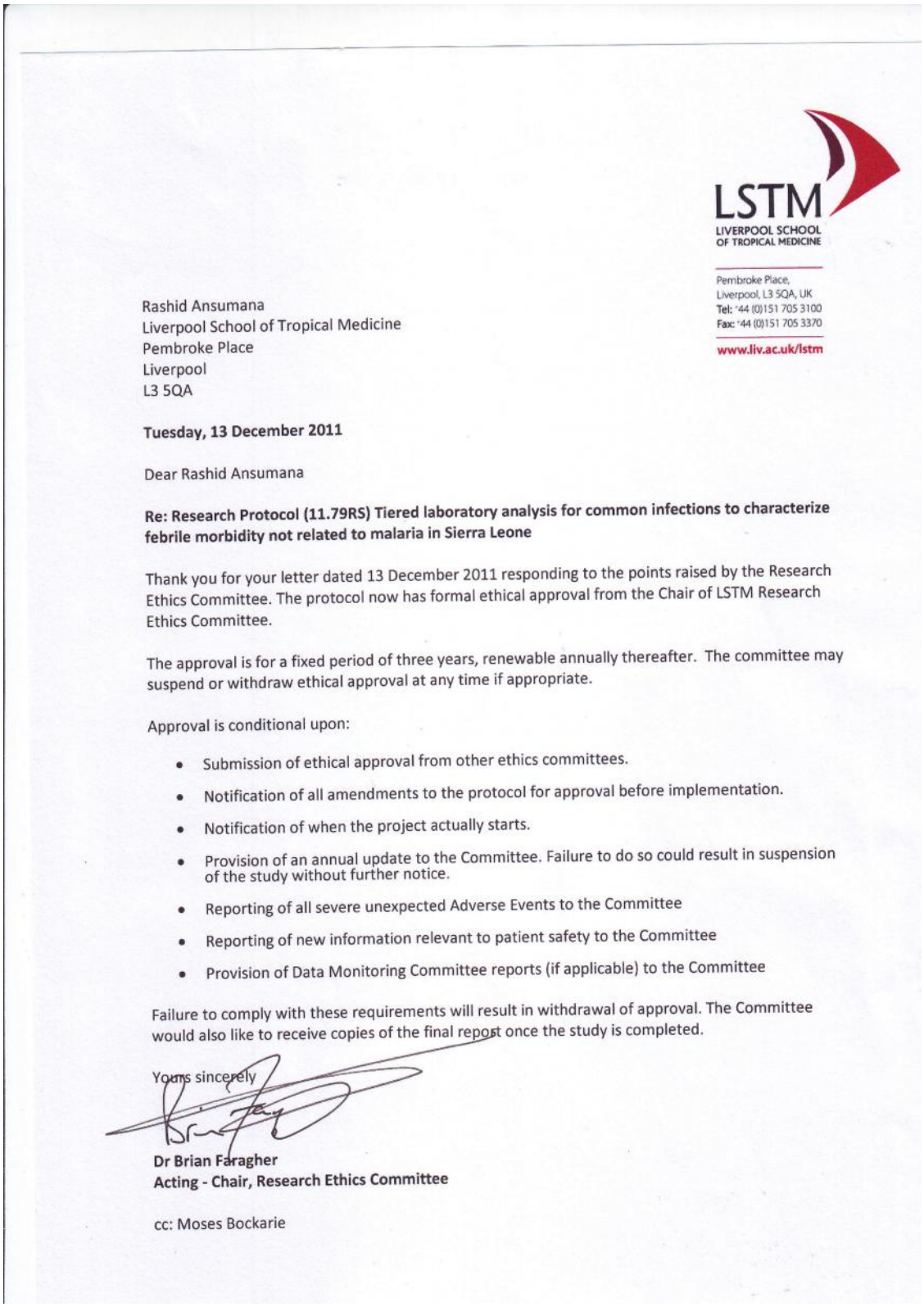
13. Ansumana R, Jacobsen KH, Bockarie MJ, Stenger DA. Point-of-care tests: where is the point? Lancet Infect Dis [Internet] 2014;14(10):922–3. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1473309914709156

14. Ansumana R, Bonwitt J, Stenger DA, Jacobsen KH. Ebola in Sierra Leone: a call for action. Lancet [Internet] 2014 [cited 2014 Oct 14];384(9940):303. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25002177

15. Ansumana R, Jacobsen KH, Sahr F, et al. Ebola in Freetown area, Sierra Leone--a case study of 581 patients. N Engl J Med [Internet] 2015;372(6):587–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25539447

APPENDICES

Appendix 1: LSTM Ethical Approval



LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44 (0)151 705 3100
Fax: +44 (0)151 705 3370
www.liv.ac.uk/lstm

Rashid Ansumana
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

Tuesday, 13 December 2011

Dear Rashid Ansumana

Re: Research Protocol (11.79RS) Tiered laboratory analysis for common infections to characterize febrile morbidity not related to malaria in Sierra Leone

Thank you for your letter dated 13 December 2011 responding to the points raised by the Research Ethics Committee. The protocol now has formal ethical approval from the Chair of LSTM Research Ethics Committee.

The approval is for a fixed period of three years, renewable annually thereafter. The committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Submission of ethical approval from other ethics committees.
- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee. Failure to do so could result in suspension of the study without further notice.
- Reporting of all severe unexpected Adverse Events to the Committee
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements will result in withdrawal of approval. The Committee would also like to receive copies of the final repost once the study is completed.

Yours sincerely

Dr Brian Faragher
Acting - Chair, Research Ethics Committee

cc: Moses Bockarie

Appendix 2: Njala University Ethical Approval

NJALA UNIVERSITY

OFFICE OF THE CHAIRMAN
INSTITUTIONAL REVIEW BOARD

18th January, 2012

Dear Mr. Ansumana

RE: Tiered Laboratory Analysis For Common Infections to Characterize Febrile Morbidity Not Related to Malaria in Sierra Leone.

The Njala University Institutional Review Board reviewed your research protocol on Monday January 16th, 2012 and the protocol has been approved. You are however required to report to the NUIRB about:

i) The date that the research starts, pauses and ends;
ii) Any amendments to your current protocol before implementation;
iii) Research related injury;
iv) Quality Control Reports and
v) Final Research Report

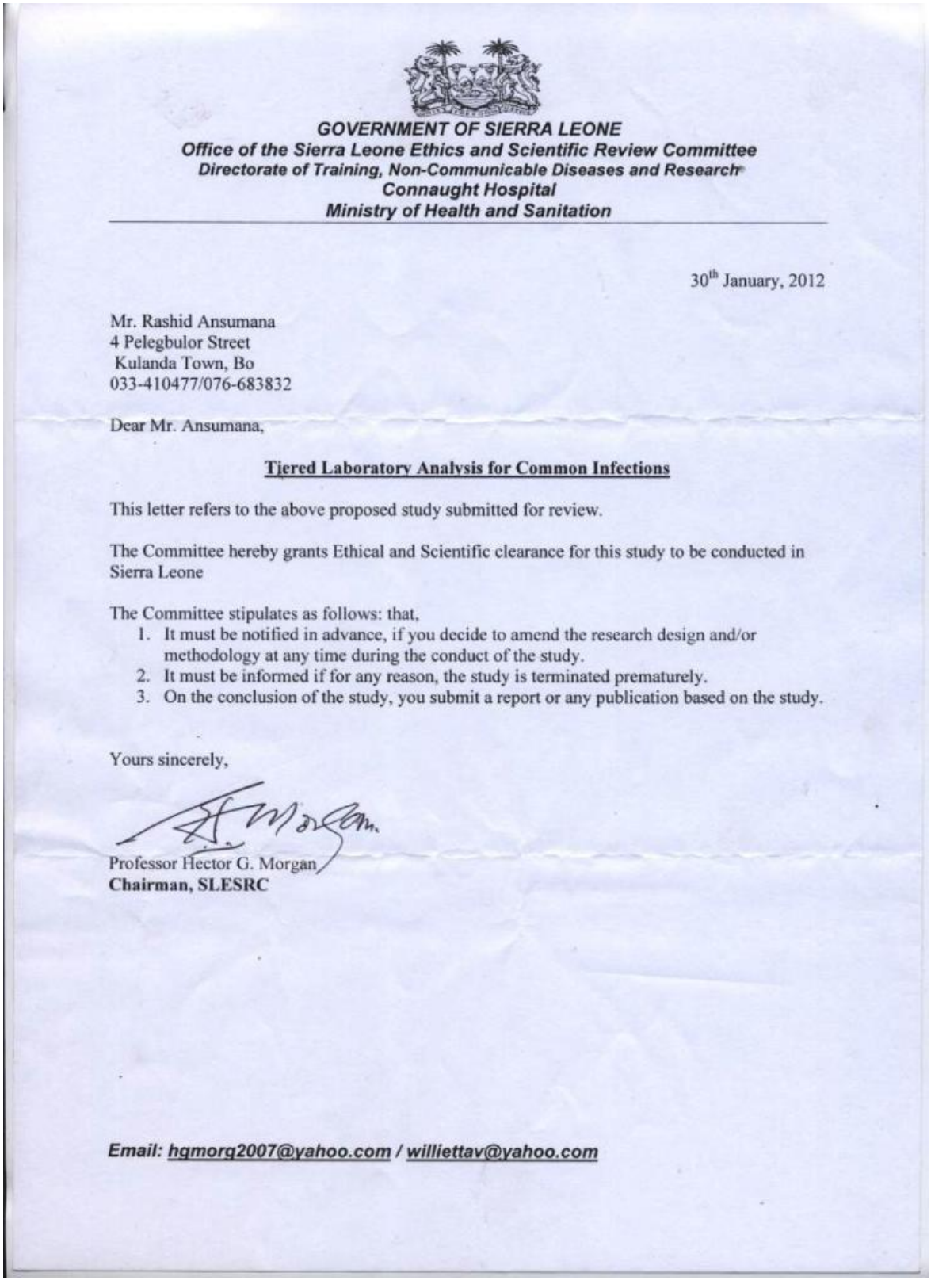
The approval is voided if you fail to comply with any of the requirements stipulated above.

Yours Sincerely

NJALA UNIVERSITY INSTITUTIONAL REVIEW BOARD
[NUIRB]
CHAIRMAN
18/01/12
DATE

Abu James Sundufu (PhD)
Chair, NUIRB

Appendix 3: Ethical Approval from the Sierra Leone Government



GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee
Directorate of Training, Non-Communicable Diseases and Research
Connaught Hospital
Ministry of Health and Sanitation

30th January, 2012

Mr. Rashid Ansumana
4 Pelegbulor Street
Kulanda Town, Bo
033-410477/076-683832

Dear Mr. Ansumana,

Tiered Laboratory Analysis for Common Infections

This letter refers to the above proposed study submitted for review.

The Committee hereby grants Ethical and Scientific clearance for this study to be conducted in Sierra Leone

The Committee stipulates as follows: that,

1. It must be notified in advance, if you decide to amend the research design and/or methodology at any time during the conduct of the study.
2. It must be informed if for any reason, the study is terminated prematurely.
3. On the conclusion of the study, you submit a report or any publication based on the study.

Yours sincerely,

Professor Hector G. Morgan
Chairman, SLESRC

Email: hgmorg2007@yahoo.com / williettav@yahoo.com

Appendix 4: Ethical Approval from George Mason University, USA

GEORGE MASON UNIVERSITY

Office of Research Subject Protections

Research Hall
4400 University Drive, MS 6D5, Fairfax, Virginia 22030
Phone: 703-993-4121; Fax: 703-993-9590

TO: Kathryn Jacobsen, College of Health and Human Service

FROM: Keith R. Bushey
Chief of Staff, Office of Research

PROTOCOL NO.: 7909 Research Category: Faculty/Staff

PROPOSAL NO.: N/A

TITLE: Tiered Laboratory Analysis for Common Infections to Characterize Febrile Morbidity not Related to Malaria in Sierra Leone

DATE: January 31, 2012

On 1/31/2012, the George Mason University Human Subjects Review Board (GMU HSRB) reviewed and approved the above-cited protocol following expedited review procedures.

Please note the following:

1. **Any modification to your research (including the protocol, consent, advertisements, instruments, funding, etc.) must be submitted to the Office of Research Subject Protections for review and approval prior to implementation.**
2. Any adverse events or unanticipated problems involving risks to subjects including problems involving confidentiality of the data identifying the participants must be reported to Office of Research Subject Protections and reviewed by the HSRB.

The anniversary date of this study is 1/30/2013. **You may not collect data beyond that date without GMU HSRB approval.** A continuing review form must be completed and submitted to the Office of Research Subject Protections 30 days prior to the anniversary date or upon completion of the project. A copy of the continuing review form is attached. In addition, prior to that date, the Office of Research Subject Protections will send you a reminder regarding continuing review procedures.

If you have any questions, please do not hesitate to contact me at 703-993-3088.

Appendix 5.0: Ethical Approval US Naval Research Laboratory

UNITED STATES GOVERNMENT

memorandum

IRB(5580)-425:MAL

DATE 07 May 2012

REPLY TO
ATTN OF: Mark A. Livingston, Code 5581

SUBJECT: New Application Approval for NRL.2012.0007, "Tiered Laboratory Analysis for Commoon infections to characterize febrile morbidity not related to malaria in Sierra Leone"

TO: David Stenger, Code 6910

REF: (a) 63 FR 216
(b) NRL Instruction 3900.2C

1. The proposed research, NRL.2012.0007, "Tiered Laboratory Analysis for Commoon infections to characterize febrile morbidity not related to malaria in Sierra Leone," has been reviewed by the HS-IRB Chair. The goal of this project is to investigate the incidence and distribution of non-malarial febrile infections in southern Sierra Leone, using tiered laboratory analyses of biosamples from febrile outpatients. Specifically, the researchers will investigate the types, etiology, and variability of non-malarial febrile illnesses in Bo, Sierra Leone (where they have previously conducted public health surveys regarding access to health care and water supply). Further, the researchers will determine the incidence of non-malarial pathogens causing febrile illnesses. Finally, they will investigate the distribution of non-malarial febrile illnesses by combining tiered analysis with a spatio-termporal febrile syndromic surveillance using a crowd-sourcing platform comprised of ushahidi and FrontlineSMS. The work is in collaboration with George Mason University and Njala University (Sierra Leone); a signed JRA covers the work.

2. Data collection through these means falls under the following category of research that may be reviewed through Expedited Procedures, as per Ref (a), Category 2: Collection of blood samples by venipuncture less than 550ml.

3. This research protocol has therefore been recommended for approval through Expedited Review by the HS-IRB Chair on 09 April 2012 and has consequently been approved by NRL's C.O. on 04 May 2012.

4. *The period of performance less than one year and expires on 30 January 2013, which corresponds with the expiration date of the collaborating institution's approval.* In accordance with Ref (b), no later than the end of this period, submit a report on subject use to the HS-IRB (form HQ-NRL 3900/2(Rev. 11-09)). Include a summary of progress to date and state any deviations from the approved protocol. *Renewals should be requested at least one month before approval expires.*

5. As required by Ref (b), NO changes may be made to the protocol or the consent form without HS-IRB re-review and approval. If adverse consequences or unexpected side effects are encountered in the course of the study, or if new information becomes available which could change the perception of a favorable risk-benefit ratio, you are responsible for informing the HS-IRB PROMPTLY. The HS-IRB must review the new information to determine if the protocol should be modified, discontinued, or should continue as originally approved.

Mark A. Livingston
Chair, HS-IRB

copy

Appendix 6: Quality Control Attestation

DEPARTMENT OF THE NAVY
NAVAL RESEARCH LABORATORY
4555 OVERLOOK AVE SW
WASHINGTON DC 20375-5320

IN REPLY REFER TO

3900
6900/086
14 Nov 2011

Prof David Lalloo
Chair, Research Ethics Committee
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool, United Kingdom
L3 5QA

Dear Sir,

I hereby write to attest that Rashid Ansumana has requested for quality control for his experiments in the project: "Tiered Laboratory Analysis of Common Infections to Characterize Febrile Morbidity Not Related to Malaria in Sierra Leone."

Our Lab shall provide the requested services for all assays that will be used in his study. We shall endeavor to present a written report on quality of his experimentation after the one-year protocol.

Please feel free to contact me for any further clarification.

V/r,

David A. Stenger, Ph.D.
CBMSE, Code 6910
U.S. Naval Research Laboratory
Washington, DC 20375
Office: 202 404 6035
Mobile: 703 786 7618
Email: David.Stenger@nrl.navy.mil

Appendix 7: Informed Consent Forms

CENTRE FOR NEGLECTED TROPICAL DISEASES - Liverpool School of Tropical Medicine

STUDY: Tiered laboratory analysis for common infections to characterize febrile morbidity not related to malaria in Sierra Leone.

Principal Investigator: Rashid Ansumana

This is a research study. Research studies include only individuals who choose to take part. Please take your time to make your decision. Discuss it with your friends and family.

You and the members of your household are being asked to take part in this study because you are in the neigbourhood of Mercy Hospital and also because you or members of your household may be at risk for or may have non-malarial febrile illnesses.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to find out the incidence and distribution of non-malarial febrile infections in Bo, Sierra Leone, using tiered laboratory analyses of blood samples from outpatients that are having fever.
This research is being done to better understand how we can prevent infection and illness from agents of nonmalarial febrile illnesses in Sierra Leone and other areas of the world where these diseases are common.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 1180 persons over 4 years from the neighbourhood of Mercy Hospital will participate in this study for blood sampling. All household heads, (about 1,027) within the neighbourhood of Mercy Hospital shall be interviewed.

WHAT IS INVOLVED IN THE STUDY?

Initially, a community survey will be done in your town section in order to obtain baseline health information about your community and to assess the occurrences of non-malarial febrile infections in your community. We will ask your permission to collect information on your property's location, and other questions pertaining to health and well-being. You will also be requested to send a text message about any febrile occurrence in your household to mobile numbers: 033-410477/076-683832

When you visit the Mercy Hospital with a febrile illness during the sampling period, you will be labeled and about 2-9mls of blood sample will be requested from you. Additionally, urine sample, stool sample or nasal swabs may be requested from you. The draw of blood may involve piercing your skin with a needle stick to obtain the blood and using pharyngeal swabs may be slightly itchy.

After we obtain the information that will allow us to determine non-malarial febrile infection and distribution, we will give this information to you and will provide treatment to those that are having non-malarial febrile illnesses who demonstrate inability to afford treatment for themselves.

HOW LONG WILL I BE IN THE STUDY?

This study will take up to one year starting in July 2012 and will continue up to July 2013.

You or your family members can decide to stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study PI first.

WHAT ARE THE RISKS OF THE STUDY?

The risk of this study is minimal. About 2-9mls of blood shall be obtained from your veins and urine sample, stool sample or nasal swabs may be requested from you. The needle stick may hurt. There is a small risk of bruising, and a rare risk of infection. Also using pharyngeal swabs may be slightly itchy.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

Your participation in this study will help to inform the appropriate persons about the incidence, cause, different types and distribution of non-malarial febrile illnesses in your community. This may help prevent infections in other members of your community and family and will also provide a guide for other communities in a similar setting.

WHAT ABOUT CONFIDENTIALITY?

Your participation in the study is confidential and your identification is anonymised. No personal information will be released to any third party unless we are required to do so by law.

WHAT ARE THE COSTS?

Taking part in the household survey and hospital sampling are of no cost to any household member. There shall be a compensation for text messages sent to inform about febrile conditions in the form of talk-time credits. In the case of injury or illness resulting from this study, emergency medical treatment shall be available at the study Hospital. 320 mg ferrous sulfate or equivalent for use, three times a day for one month will be available for subjects that are anaemic.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose to remove your home from the study at any time. Leaving the study will not result in any penalty. However, if you decide to stop participating in the study, we encourage you to talk to the study PI first.

HOW WILL I KNOW THE RESULTS OF THE STUDY?

We will tell you/your household members about new information that may affect your health, welfare, or willingness to stay in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact: Rashid Ansumana (+23233-410477/+23276-683832) or Edries Tejan (+23233-313375) at Mercy Hospital and Research Laboratory, Kulanda Town Bo; or the Sierra Leone Ethics and Scientific Review Committee (+23276463696) at the Directorate of Training, Non-Communicable Diseases and Research, Connaught Hospital, Freetown.

CONSENT OF HEAD OF HOUSEHOLD.

You must be an adult (age 18 or older) to answer the survey questions and head of household to consent here for participation in the survey. Your wards must also be aged 5 or older to be a subject for sampling in the hospital when they have fever. You must be a mother, father or guardian to grant assent for minors. If you agree to participate or your ward to participate in the entire study including responding to the questionnaire, providing blood, urine, stool samples or pharyngeal swabs and sending text messages about fever in your household please sign below:

Name (printed) Signature or thumbprint
Date

_____

ASSENT OF HOUSEHOLD MEMBERS

Name (printed) Signature or thumbprint
Date

_____

Name (printed) Signature or thumbprint
Date

_____

Name (printed) Signature or thumbprint
Date

_____

ASSENT OF HOUSEHOLD MEMBERS (SUPPLEMENTARY)

Name of Minor (if applicable)	Thumbprint	Date
Name of Minor (if applicable)	Thumbprint	Date
Name of Minor (if applicable)	Thumbprint	Date
Name of Minor (if applicable)	Thumbprint	Date
Name of Minor (if applicable)	Thumbprint	Date
Name of Minor (if applicable)	Thumbprint	Date

KRIO VERSION OF INFORMED CONSENT

Stɔdi: Fɛnɔt wok bɔt dɛn kɔmɔn wam bɔdi sik we nɔ mix wit maleria we kin kil pipul dɛm na salon

Di Men Pɔsin We De Du De Fɛnɔt: Rashid Ansumana

Dis na fɛnɔt wok. Fɛnot wok jis gɛt pipul dɛm we want fɔ do di wok. Duya tek yu tɛm fɔ disaid. Tɔkam wit you padi dɛm ɛn yu fambul dɛm.

Yu ɛn dɛn wan dɛm we tap na yu os fɔ de pan dis wok bikɔs una de nia Maci Ɔspitul ɛn i pɔsibul se una go kech dɛn wam bɔdi sik we nɔ mix wit maleria

Wetindu Dis Wok De Bi?

Dis wok na fɔ fɛnɔt aw dɛn sik we de mek yu bɔdi wam we nɔ mix wit maleria de kech pipul dɛn na Bo, insai salon, ɛn na blɔd sampul wi de yus from pipul dɛm we nɔ admit yet fɔ dɛn sik dɛm. Dis fenɔt wok nafɔ mek wi no gud gud wan aw fɔ tap dɛn wam bɔdi sik dɛm we nɔ mix wit maleria from ɛni sai we wi kin get dɛm na salon ɛn ɔda sai dɛm nadiwɔl.

Ɔmɔs Pipul Dɛm Go De Pan Dis Wok?

Na bɔt wan tawzin, wan undrɛd ɛn eti(1180) pipul dɛm oba fo(4) yia we tap na di eria we Masi Ɔspitul de, go de pan dis blɔd sampul wok. ɔl os edman dɛm (bot wan tawzin ɛn twɛnti sɛvin-1027) pan dɛm pipul dɛm we tap na masi ɔspital eria go ansa quɛshɔn dɛm.

Wetin Dem De Pandis Wok?

Fɔs we go fɛnɔt na u eria fɔ no di wɛlbodi biznɛs bɔt aw dɛn wam bɔdi sik we nɔ mix wit maleria de kech pipul dɛm de. We go beg padin fɔ no usai yu os de wit GPS ɛn sem we so, we go ax bɔt welbodi biznes. Wi go ax yu iƉŋen fɔ sɛn tɛx bɔt ɛni wam bɔdi sik we no mix wit maleria we de na yu eria, na dis number: 033-410477/076-683832.

Di tɛm we di blɔd sampul de keri on, if yu sik with wam bɔdi yu go Masi Ɔspitul, dɛn go ax fɔ pul yu blɔd smɔl, lek nain mil(9mls). We go us nidul fɔ pul di blɔd. We wi dɔn fɛnɔt, wi go no gud gud wan bɔt dɛn sik we nɔ mix wit maleria. We go tel yu ɛn wi go mɛn de wan dɛm we nɔ ebul gɛt mɔni fɔ mɛn dɛnsɛf.

Aw Lɔnŋŋ Ago De Pan Dis Wok?

Dis wok go de sote wan yia, bigin frɔm Januari, 2012 ɛn tap Disɛmba 2012. Yu ɔ yu fambul dɛm kin disaid fɔ lɛf dis wok ɛni tɛm una want. But if yu want fɔ lɛf, duya mek lɛ di men pɔsin we de du di fɛnɔt no se yu de lɛf.

Wetin Na Di Ɛngat Tin Dɛm Bɔt Dis Wok?

Di ɛngat tin dɛm nɔ bɔku. Na smɔl blɔd, bɔt nain mil(9mls)

nɔ mɔ wi de pul pan yu, na yu vɛn. Di nidul go at smɔl. Na smɔl pinch nɔmɔ dat go do pan yu bɔdi ɛn yu no go ɛngat se yu go get ɛni sik.

Us Bɛtɛ Tin Dɛm De Pan Dis Wok?

Yu de wan pan dis wok go mek di big eleju dɛm no bɔt dɛn sik we de mek pɔsin ɛn bɔdi wam, we nɔ to maleria na yu eria. Dis go ep tap dɛn kain sick dɛm na ɔda eria dɛm we fiba usai u de.

Aw Una De Kip Tin Dem Sikrit?

Wi go trai fɔ kip sikrit ɛnitin we na bɔt yu nɔmɔ, ɛn nobɔdi nɔ go no ɛnitin bɔt yu ɔ yu pipul dɛm. ɛniwe, wi nɔ go tel yu se ɔltin go bi sikrit, bicɔs if di lɔ ax wi fɔ sho wetin we gɛt bɔt yu, wi go duam.

Ɔmɔs Ibi Fɔ De Pan Dis Wok?

Nobɔdi no go pay wan sɛnt fɔ de pan dis wok. Smɔl kɔpɔ fɔ tɔp-ɔp go de fɔ dɛn tɛx mɛseg dɛm we yu go sɛn bɔt dɛn wam bodi sik dɛm na yu ose. If yu wund ɔ sik fɔ seka dis wok, di ɔspitul go mɛn yu kwik kwik wan. 320mg blɔd mɛrɛsin, tri tɛm fɔ de, fɔ wan mɔnt go de fɔ ɛnibɔdi we in blɔd lo, wans yu de pan dis wok.

Wetin Na Mi Rait As Posin We De Pan Dis Wok?

Fɔ de pan dis wok nɔto baifɔs, na we yu lek. If yu want, yu kin pul yu fambul dɛm pan dis wok ɛni tɛm ɛn yu nɔgo gɛt ɛni problɛm. Bɔt ɛni we, if yu disaid fɔ lɛf, du ya na fɔ tɛl di men pɔsin fɔ dis fɛnɔt .

Aw Ago No Wetin Kɔmɔt Pan Dis Fɛnɔt?

We go tɛl yu ɔ yu pipuldɛm bɔt niu tin dɛm we go gɛt fo du sɔmtin wit yu welbɔdi wan o yu gri wan fo de pan dis wok.

Udat A Go Cɔl If A Wan Ax Sɔmtin Ɔ A Get Prɔblem?

Fɔ ax ɛni tin bɔt dis wok we dis fɛnɔt wok de bɔt, na fo si: Rashid Ansumana, Masi Ɔspitul, fɛnɔt wok sai, Kulanda tɔn na Bo, yu kin kɔlam na +23233-410477/+23276-683832

Fɔ Mɛk Yu No.

Yu fɔ bi big pɔsin we ole tin(18) yia ɔ oba fɔ ansa de fɛnɔt kuɛshon dɛm ɛn yu fɔ ol faiv yia o oba fɔ gi blɔd, ɛn mama, papa ɔ yu we de mɛn pikin fɔ gri fɔ dɛn smɔl pikin dɛm fɔs. If yu gri fɔ de pan dis wok o yu pikin duya sain bɔttɔm

Nem sain/tɔmbprint Det

Nem fɔ smɔl piki tɔmbprint Det

(if dat de wok)

Appendix 8: Clinical Addendum

CENTRE FOR NEGLECTED TROPICAL DISEASES - Liverpool School of Tropical Medicine

STUDY: Tiered laboratory analysis for common infections to characterize febrile morbidity not related to malaria in Sierra Leone.

Principal Investigator: Rashid Ansumana

A. HOW TO DETERMINE SUBJECTS THAT CANNOT AFFORD TO PAY

The method used at Mercy Hospital to determine those that cannot afford to pay include:

1. Subjects that are aged, impoverished; who present unwell, and cannot pay for their treatments

2. Subjects that have in previous months before the onset of the research, being unable to pay for their treatments

3. Those subjects that lack all the money needed to purchase prescribed drugs at the pharmacy

4. Inpatients, that are subjects, without family to assist them during their admission and/ without employment and without money to pay for their treatment costs.

B. MONETARY CEILING FOR TREATMENT OF DESTITUTE SUBJECTS

For outpatients at Mercy Hospital, about 13 destitute persons were treated in January 2012 and none in February 2012. The average cost of treatment provided to destitute outpatients in January was Le 29,000 (about 7 USD). For inpatients in January, 7 were present, the average cost was Le 96, 428 (22 USD). In February, there was no destitute outpatient or inpatient. Based on the January data, the following monetary ceiling has been considered for destitute subjects in the form of drugs that are prescribed and are available at the hospital.

1. A ceiling of 7 USD for outpatients that satisfy A. 1, 2 and 3 above

2. A ceiling of 22 USD for inpatients that satisfy A. 1-4.

3. Monthly ceiling of 200 USD for both outpatients and inpatients.

Please note below that:

1. While treatment is been provided for destitute subjects, only treatment that are stipulated above will be provided.

2. The cost stipulated above is the ceiling. Outpatients and inpatients that cannot afford to pay will be treated for less if that is what their condition demands.

C. SIGNATURE

The patient/authorized individual has read this form or had it read to him or her.

The patient/authorized individual states that he or she understands this information.

The patient/Authorized individual has no further questions.

Date_____ Time_____ Signature_____

Appendix 9: SYNDROMIC STUDY QUESTIONAIRE

Household Address: _____

Household ID: _____

SOCIO-DEMOGRAPHIC INFORMATION

How many households (families) currently live in this house? _____

How many people currently live in this household (family)? _____

Only count people who spend at least half of the year at this residence. Exclude students at boarding school and adults who spend most of their time working in another town.

Personal ID#	Relationship to Head of Household	Sex (M/F)	Age (years)	Under-5 (U5) or Mother (M)?
01	Head of Household			
02				
03				
04				
05				
06				
07				
08				

09				
10				
11				
12				
13				
14				
15				
16				

→ *Put a star (*) next to the person answering this survey. Use a continuation page if needed.*

How many people (adult or child) who were living in this household one year ago (or born in the past year) died in the past one year? _____

	Age	Sex (M / F)	Cause of Death
1			
2			
3			

Use a continuation page if needed.

HOUSING AND UTILITIES

1. What is the primary material used for the **construction** of the house?

☐ Concrete block ☐ Mud and sticks ☐ Mud block ☐ other: _____

2. What is the primary material used for the **roof** of the house?

☐ Zinc ☐ Thatch ☐ Tarpaulin ☐ other: _____

3. What is the main floor material used in the house?

☐ covered with floor tile☐ Concrete floor ☐ Muddy floor ☐ Other: _____

4. Does the house have electricity? ☐ Yes ☐ No

5. Do the beds in the household have mosquito nets? ☐ Yes ☐ No

6. What is your primary source of water for drinking?

☐ Well ☐ Stream / River ☐ Spring ☐ Standpipe / Piped water

☐ Enclosed pump ☐ Bottled water / Pure water

7. Does your household use the same source for drinking water and cooking water?

☐ Yes ☐ No ☐ Not sure

8. How close to your home is your source of drinking water?

☐ <50 meters ☐ 50-150 meters ☐ 151-500 meters ☐ >500 meters

9. Do you have any trashcan in your household ☐ Yes ☐ No

10. If yes, is it covered? ☐ Yes ☐ No

11. What type of toilet facility is used by your household?

☐ Bucket toilet ☐ Flush latrine ☐ Pit latrine

☐ Uncovered pit☐ other:

12. If applicable, about how frequently do you empty your toilet? ☐ Not applicable

☐ 1 time per day ☐ 1 time each month ☐ 1 time per year

☐ 1 time every few years ☐ other:

_____

13. If applicable, who does the emptying of the toilet?

☐ Household member ☐ Hired worker ☐ Other: _____

14. How many rooms in your house are used for sleeping? _____

15. How many people usually sleep in this house each night? _____

16. In the past month, did you see any cockroach in your house? ☐ Yes ☐ No

17. In the past month, did you see any rat in your house? ☐ Yes ☐ No

18. Do you have a pet in your house? ☐ Yes ☐ No

19 What type of animal(s) do you have as a pet?_____

HOUSEHOLD FEBRILE INFORMATION

20. Has anyone currently living in this household had malaria in the past one month?

☐ No ☐ Yes ☐ Not sure

21. If yes, was the person(s) diagnosed in a hospital? ☐ No ☐ Yes

22. If more than one person in this household had malaria, state:

Number tested in a lab_____ Number not tested_____

23. Has anyone currently living in this household ever had fever in this year?

☐ Yes ☐ No

24. If yes, how many times did the household member have fever in the past months of this year?

Member ID_____ No of Fever in this year_____ Approx. time of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

Member ID_____ No of Fever in this year_____ Approx. times of fever:
☐ 1 Month or Less ☐ 2 months or Less ☐ 3 months or Less ☐ Over 4 months

26. Was the fever without any localized source of infection? ☐ No ☐ Yes

27. Was the fever of 14 days or less in duration? ☐ No ☐ Yes

28. Was the fever, with a symptom or sign? ☐ No ☐ Yes

29. Was it (a fever–arthralgia?), a fever and tenderness over three or more joint areas? ☐ No ☐ Yes

30. Was it a fever (fever–myalgia syndrome?): fever with body ache or headache?

☐ No ☐ Yes

31. Was it a fever with altered behaviour? ☐ No ☐ Yes

32. Was it a fever with jaundice? ☐ No ☐ Yes

33. Was any test done to find out the cause of the fever? ☐ No ☐ Yes

34. If yes, what was the cause of the fever?_____

35. If no, was clinical examination done? ☐ No ☐ Yes

36. Did the household member regain his/her health? ☐ No ☐ Yes

37. Has anyone currently living in this household ever had any of the following conditions?

Hepatitis B	☐ No	☐ Yes	☐ Not sure
Hepatitis C	☐ No	☐ Yes	☐ Not sure
Typhoid Fever	☐ No	☐ Yes	☐ Not sure
Lassa Fever	☐ No	☐ Yes	☐ Not sure
Yellow Fever	☐ No	☐ Yes	☐ Not sure
Pneumonia	☐ No	☐ Yes	☐ Not sure
Influenza	☐ No	☐ Yes	☐ Not sure
Common Cold	☐ No	☐ Yes	☐ Not sure
Asthma	☐ No	☐ Yes	☐ Not sure
TB	☐ No	☐ Yes	☐ Not sure
Rift valley fever	☐ No	☐ Yes	☐ Not sure
Crimean-Congo hemorrhagic fever	☐ No	☐ Yes	☐ Not sure
Dengue fever	☐ No	☐ Yes	☐ Not sure
Bacterial meningitis	☐ No	☐ Yes	☐ Not sure

Others
Specify_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Appendix 10: LIST OF MANSUCRIPTS PUBLISHED BETWEEN 2010-2015 (STUDY PERIOD)

Dziedzom K. de Souza, **Rashid Ansumana**, Santigie Sessay, Abu Conteh, Benjamin Koudou, Maria P. Rebollo, Joseph Koroma, Daniel A. Boakye, Moses J. Bockarie. The impact of residual infections on Anopheles-transmitted Wuchereria bancrofti after multiple rounds of mass drug administration. Sept. 2015. *Parasites and Vectors* (*in press*)

Roger Hillson, Joel D. Alejandre, Kathryn H. Jacobsen, **Rashid Ansumana**, Alfred S. Bockarie,Umaru Bangura,Joseph M. Lamin, David A. Stenger.Stratified Sampling of Neighborhood Sections for Population Estimation: A Case Study of Bo City, Sierra Leone. PLoS One. 2015 Jul 15;10(7):e0132850. doi: 10.1371/journal.pone.0132850. **3.53 Impact Factor.**

Tomasz A Leski, **Rashid Ansumana**, Chris R. Taitt, Matt Scullion, Joseph M. Lamin, Umaru Bangura, Joseph Lahai, George Mbayo, Mohamed B. Kanneh, Ben Bawo[2], Alfred S. Bockarie, David Stenger. . Use of FilmArray™ system for detection of Zaire ebolavirus in a small hospital, Bo, Sierra Leone(J. Clin. Microbiol. 2015 May 13)

Shamika Ranasinghe,[a] **Rashid Ansumana**,[b,c] Joseph M. Lamin,[b] Alfred S. Bockarie,[b] Umaru Bangura,[b] Jacob A.G. Buanie,[b] David A. Stenger,[d] and Kathryn H. Jacobsen[a.]

Herbs and herbal combinations used to treat self-diagnosed malaria in Bo, Sierra Leone (*Ethnopharmacology, 2015).*

Shamika Ranasinghe, **Rashid Ansumana**, Joseph M. Lamin, Alfred S. Bockarie, Umaru Bangura, Jacob A.G. Buanie, David A. Stenger and Kathryn H. Jacobsen. Attitudes toward home-based malaria testing in rural and urban Sierra Leone.**(***Malaria Journal,2015***)**

Hannah Brown, Ann H. Kelly, Almudena Mari-Saez, Elisabeth Fichet-Calvet, Stephan Günther , **Rashid Ansumana**, N'Faly Magassouba , Foday Sahr, Matthias Borchert. Extending the 'social': Anthropological contributions to the study of viral haemorrhagic fevers. (*PLOS NTD,2015*).

Tomasz A. Leski, Michael G. Stockelman, Lina M. Moses, Matthew Park , David A. Stenger, **Rashid Ansumana**, Daniel G. Bausch, Baochuan Lin. Sequence Variability and Geographic Distribution of Lassa Virus in Sierra Leone. *(EID).*

Rashid Ansumana, Kathryn H Jacobsen, M'baimba Idris, Henry Bangura, Mohamed Boi-Jalloh, Joseph M. Lamin, Santigie Sesay, and Foday Sahr. Ebola in Freetown Area, Sierra Leone: A Case study of 581 patients. NEJM Dec. 24, 2014)

Roger Hillson, Joel Alejandre, **Rashid Ansumana**, Kathryn H Jacobsen,Alfred S. Bockarie[3] Anthony Malanoski, Umaru Bangura, Joseph M. Lamin, David A. Stenger Methods for Determining the Uncertainty of Population Estimates Derived from Satellite Imagery and Limited Survey Data: A Case Study of Bo City, Sierra Leone Survey Data. PLoS ONE 9(11): e112241.

Abu James Sundufu, **Rashid Ansumana**, Alfred S. Bockarie, Umaru Bangura, Joseph M. Lamin, Kathryn H. Jacobsen, and David A. Stenger. Syndromic surveillance of livestock disease incidence and mortality in Koinadugu District, Sierra Leone, 2011-2012.*Journal of Tropical Animal Health and Production, 12/2014;46(8).*

Rashid Ansumana, Jesse Bonwitt, David A. Stenger, Kathryn H Jacobsen. Ebola in Sierra Leone: a call for action. . The Lancet, Volume 384, Issue 9940, Page 303, 26 July 2014.

Rashid Ansumana, Kathryn H Jacobsen, Moses J Bockarie, David A. Stenger. Point of Care tests: where is the point? Lancet Infectious Diseases, 10/2014; 14(10):922–923.

Mackenzie Dome[*], **Rashid Ansumana**[*&], Andrea L. Covington, Maria Rebollo, Santigie Sesay, Kathryn H. Jacobsen, *Dziedzom De Souza, Benjamin Koudu,Edwin Michael And Moses J. Bockarie. A Case of Filarial Lymphedema in A 7 Year Old Boy in Sierra Leone. 2014.* **Acta Tropica** .

Dziedzom K. de Souza[1], Santigie Sesay, Marnijina G. Moore[3], **Rashid Ansumana**, Karsor Kollie, Maria Rebollo, Benjamin G. Koudou, Joseph B. Koroma, Fatorma K. Bolay, Daniel A. Boakye and Moses J. Bockarie. No evidence for lymphatic filariasis transmission in big cities affected by conflict related rural-urban migration in Sierra Leone and Liberia. *2014. PLOS NTD.*

Bhoobun S, Jetty A,Koroma MA, Kamara MJ, Kabia M, Coulson R, **Ansumana R**,Jacobsen KH. Facilitators and barriers related to voluntary counseling and testing for HIV among young adults in Bo, Sierra Leone. *J Community Health. Nov. 2013.*

Leski TA, Bangura U, Jimmy DH, **Ansumana R**, Lizewski SE, Stenger DA, Taitt CR, Vora GJ. Multidrug-resistant tet(X)-containing hospital isolates in Sierra Leone. *International Journal of Antimicrobial Agents.* 2013. *Epub ahead of print.*

Leski TA, Bangura U, Jimmy DH, **Ansumana R**, Lizewski SE, Li RW, Stenger DA, Taitt CR, Vora GJ. Identification of blaOXA-51-like, blaOXA-58, blaDIM-1 and blaVIM carbapenemase genes in hospital Enterobatereriaceae isolates from Sierra Leone. *Journal of Clinical Microbiology*. 2013. *Epub ahead of print.*

Ansumana R, Jacobsen KH, Leski TA, Covington AL, Bangura U, Hodges MH, Lin B, Bockarie MJ, Stenger DA. Reemergence of chikungunya virus in Bo, Sierra Leone. *Emerging Infectious Diseases.* 2013 Jul; 19(7):1108-1110.

Ansumana R, Jacobsen KH, Gbakima A, Hodges MH, Lamin JM, Leski TA, Malanoski AP, Lin B, Bockarie MJ, Stenger DA. Presumptive self-diagnosis of malaria and other febrile illnesses in Sierra Leone. *Pan African Medical Journal.* 2013 May 26; 15:34.

Ansumana R, Bockarie AS, Sankoh P, Jacobsen KH, Koroma AB, Malanoski AP, Meehan KA, Leski T, Jimmy DH, Bangura U, Sahr F, Lin B, Stenger DA. The use of mobile electronic devices for public health data collection and syndromic surveillance in Africa. *Sierra Leone Journal of Biomedical Research*. 2013 Apr; 5(1):8-14.

Jimmy DH, Sundufu AJ, Malanoski AP, Jacobsen KH, **Ansumana R**, Leski TA, Bangura U, Bockarie AS, Tejan E, Lin B, Stenger DA. Water quality associated public health risk in Bo, Sierra Leone. *Environmental Monitoring and Assessment*. 2013 Jan; 185(1):241-251.

Jacobsen KH, **Ansumana R**, Abdirahman HA, Bockarie AS, Bangura U, Meehan KA, Jimmy DH, Malanoski AP, Sundufu AJ, Stenger DA. Considerations in the selection of healthcare providers for mothers and children in Bo, Sierra Leone: reputation, cost, and location. *International Health*. 2012 Dec; 4(4):307-313.

Leski TA, **Ansumana R**, Malanoski AP, Jimmy DH, Bangura U, Barrows BR, Alpha K, Koroma BM, Long NC, Sundufu AJ, Bockarie AS, Lin B, Stenger DA. Leapfrog diagnostics: demonstration of a broad spectrum pathogen identification platform in a resource-limited setting. *Health Research Policy and Systems*. 2012 Jul 4; 10:22.

Jacobsen KH, Abdirahman HA, **Ansumana R**, Bockarie AS, Bangura U, Jimmy DH, Malanoski AP, Sundufu AJ, Stenger DA. Home birth and hospital birth trends in Bo, Sierra Leone. *Acta Obstetricia et Gynecologica Scandinavica*. 2012 Jun; 91(6):750-753.

Taitt CR, Leski T, Stenger DA, Vora GJ, House B, Nicklasson M, Pimental G, Zurawski DV, Kirkup BC, Craft D, Waterman PE, Lesho EP, Bangura U, **Ansumana R**. Antimicrobial resistance determinant microarray for analysis of multi-drug resistant isolates. *SPIE Proceedings*. 2012 May 1; 8371. doi:10.1117/12.924569

Jacobus H, Lin B, Jimmy DH, **Ansumana R**, Malanoski AP, Stenger DA. Evaluating the impact of adding energy storage on the performance of a hybrid power system. *Energy Conversion and Management*. 2011 Jul; 52(7):2604-2610.

Leski TA, **Ansumana R**, Jimmy DH, Bangura U, Malanoski AP, Lin B, Stenger DA. Massively multiplexed microbial identification using resequencing DNA microarrays for outbreak investigation. *SPIE Proceedings*. 2011 Apr 25; 8029. doi:10.1117/12.884782

Meehan KA, Bankoski AJ, Tejan E, **Ansumana R**, Bangura U, Stenger DA, Jacobsen KH*. Hypertension in Bo, Sierra Leone. *Ethnicity & Disease*. 2011 Spring; 21(2):237–242,259.

Ansumana R. 2011. Use of fast technology for the analysis of nucleic acids to store ribonucleic

acid extracts at elevated temperatures. *International Journal of Collaborative Research on Internal Medicine & Public Health* 04/2011; 3(3):261-261.

Ansumana R, Malanoski AP, Bockarie AS, Sundufu AJ, Jimmy DH, Bangura U, Jacobsen KH, Lin B, Stenger DA. Enabling methods for community health mapping in developing countries. *International Journal of Health Geographics*. 2010 Oct 29; 9:56.

PanAfrican Medical Journal

Research

Presumptive self-diagnosis of malaria and other febrile illnesses in Sierra Leone

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Key words: Malaria, fevers, self-care, health services accessibility, community pharmacy services, West Africa

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Abstract

Introduction: The objective of this study was to evaluate the prevalence of self-diagnosis of malaria and other febrile illnesses in Bo, Sierra Leone. Methods: All households in two neighboring sections of Bo were invited to participate in a cross-sectional survey. Results: A total of 882 households (an 85% participation rate) that were home to 5410 individuals participated in the study. Of the 910 individuals reported to have had what the household considered to be malaria in the past month, only 41% were diagnosed by a healthcare professional or a laboratory test. Of the 1402 individuals reported to have had any type of febrile illness within the past six months, only 34% had sought a clinical or laboratory diagnosis. Self-diagnosis of influenza, yellow fever, typhoid, and pneumonia was also common. Conclusion: Self-diagnosis and presumptive treatment with antimalarial drugs and other antibiotic medications that are readily available without a prescription may compromise health outcomes for febrile adults and children.

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Introduction

The overuse or inappropriate use of antimalarial medications and antibiotics is a growing concern in many parts of the world, including Sierra Leone, a post-conflict country in West Africa. In Sierra Leone, these drugs are dispensed by government-run clinics and hospitals, private healthcare facilities, licensed pharmacies, and through the informal sector [1]. Because medications are readily available without a prescription, people with self-diagnosed infections can access treatments without first seeking a formal clinical consultation and/or laboratory-confirmed diagnosis. A survey conducted in April 2009 in several parts of Sierra Leone — including the city of Bo, the focus of this paper — found that 50.8% of anti- malarial drugs were dispensed without a prescription [1].

Easy access to antimalarial drugs and other antibiotic agents may compromise patient health outcomes. If the medications purchased for an infection are inappropriate — such as antibiotics taken for a viral infection or chloroquine taken for a chloroquine-resistant case of malaria — or if medications are not taken in the appropriate dosage and for an appropriate length of time, self-diagnosis and treatment may contribute to prolonged illness, more severe morbidity, and an increased risk of mortality. A study in Bo district in 2008 found that only 48.3% of malaria-positive patients at a hospital that offered free care completed the full course of prescribed antimalarial treatment [2]. Given that low rate of adherence to prescribed treatment regimens, it is likely that few patients who access over-the-counter antibiotics complete a full course of an appropriate, government-recommended formulation. The antimalarial medications available over-the-counter are rarely the formulations recommended by the national government and the World Health Organization (perhaps a good sign that national health system drugs are not leaking into the private market), and the 2009 study found a very low level of knowledge about national and international antimalarial policies and regulations among private- sector sellers of medication, compared to a high level of knowledge among public-sector providers [1].

Furthermore, misuse of antimicrobial agents can contribute to the emergence of drug resistance, which is a growing concern in Sierra Leone. A 2002-2003 study found treatment failure among more than half of pediatric malaria patients treated with chloroquine, and treatment failure was also frequently observed for sulphadoxine-pyrimethamine (SP) [3]. These results suggest that drug-resistant malaria is already common in the study area. Evidence of drug- resistant bacterial infections has also been reported, including outbreaks of multidrug-resistant *Shigella dysenteriae* in 1999-2000 [4] and drug resistant *Staphylococcus aureus* among children during a 2008-2009 study in Freetown [5]. Sierra Leone has one of the highest rates of multidrug-resistant tuberculosis (MDR-TB) in sub- Saharan Africa [6].

Over-diagnosis of malaria, in particular, may be common. Even clinicians can find it difficult to diagnose malaria accurately solely based on symptoms. A study in the Bo district in 2005 found that 82% of children suspected by clinical officers to have malaria based on physical symptoms such as fever, splenomegaly, and vomiting tested positive for parasitemia by a Paracheck-pf® rapid diagnostic test (RDT) and 18% did not [7]. Between 2004 and 2006 in the same region of Sierra Leone, only 65% of the Paracheck® RDTs performed on pediatric patients with clinically — suspected malaria were positive, suggesting that antimalarial medication may have been significantly over-prescribed without the use of confirmatory laboratory tests [8]. It is important to note that some children in highly-endemic areas test negative for malaria at the beginning of a febrile illness, such as a case of pneumonia, but then become malaria-positive later in their course of illness due to their weakened state. (Additionally, many children test positive for malaria even when they are asymptomatic.) Parents without clinical training might be more likely than healthcare professionals to diagnose any febrile illness as malaria and to seek presumptive antimalarial treatment. While presumptive treatment may be helpful — and perhaps even lifesaving — when the child actually does have malaria, a misdiagnosis may result in delayed treatment for the actual cause of the fever, and the delay in seeking professional medical care may increase morbidity and mortality [9]. The goal of this paper was to evaluate the prevalence of self-diagnosis of malaria and other febrile illnesses in Bo, Sierra Leone's second largest city.

Methods

Sampling Strategy. All households within the Kulanda Town and Njai Town sections (neighborhoods) of the city of Bo were eligible for participation. The research laboratory has previously mapped all of the buildings within these sections and conducted a household census, in 2010, to identify which structures were residential ones [10]. The resulting geographic information system (GIS) was used to create a map of all of the homes within the study community. In June 2012, members of the research team visited each household on the map, as well as 11 new households (which were added to an updated map), to ask for their participation. Of the 1038 households in these sections, 882 (85.0%) agreed to participate. For each of these households, one adult, usually the head of the household, was interviewed to gather information about the whole household.

Data Collection. Each interview began with questions about the household's environmental characteristics, such as building materials and access to utilities, and about household demographics, such as the age and sex of each current resident. Students at boarding school, adults working in another town and not sleeping at the residence in Bo, and others who spent at least 6 months of the past year living elsewhere were not considered to be current household members.

Then a series of questions were asked about febrile illnesses experienced by household members. These questions were developed in consultation with residents of the Kulanda Town / Njai Town sections to ensure clarity, and asked about all household members of all ages. First, the household representative was asked whether anyone currently living in the household had been ill with what the household considered to be malaria in the past one month. If malaria was reported to have occurred, follow-up questions asked where those with malaria were diagnosed (such as at a hospital or clinic, at home by a nurse, or at home by an untrained person — that is, self-diagnosis) and whether they were tested for malaria by a laboratory. A second set of questions asked whether anyone currently living in the household had a febrile illness earlier in 2012 (that is, in the 6 months prior to the interview). Follow-up questions asked about the frequency and duration of febrile illnesses; symptoms associated with these fevers (such as joint tenderness, headaches, altered behavior, and jaundice); and whether the febrile person was examined by a clinician and/or had a laboratory test to determine the cause of the fever. A final set of yes/no questions asked whether the household considered anyone currently living in the household to have ever had any of more than a dozen listed communicable and non-communicable conditions, and whether those diagnoses were made by a doctor or were self-diagnosed.

Data Management and Analysis. Responses were entered by the interviewers directly into a Filemaker Pro 12 relational database

on a password-protected tablet computer. Households were identified by a number linked to a map stored at the research laboratory; these codes were random and not related to the geographic coordinates of the map. Data were analyzed using the statistical software program SPSS (version 20). Proportions, means, and standard deviations were used to describe the variables. Chi- squared tests were used to compare rates in independent populations, such as different age groups.

Ethical Considerations. Adults ages 18 and older were interviewed after providing informed consent, which was documented with a signature or a thumbprint. The consent form and study materials were available in both English and Krio, the local language in Sierra Leone. No compensation or other incentive was offered. To protect the confidentiality of information shared with the research team, no names or addresses were entered into the database. The data entered into each tablet computer were deleted daily after the data files on the tablets were downloaded to a password-protected desktop computer in a locked and guarded research facility. The research protocol was approved by the Sierra Leone Ethics and Scientific Review Committee and by Njala University (Sierra Leone), the Liverpool School of Tropical Medicine (UK), the U.S. Naval Research Laboratory (USA), and George Mason University (USA).

Results

The 882 participating households contained 5410 individuals, with a mean household size of 6.1 persons. The households reported a somewhat diverse set of socio-environmental characteristics. While 1186 (66.7%) of the 1778 beds reported to be located in the participating homes were said to have bednets, 328 (37.2%) of households reported having no bednets. In total, 877 (99.4%) of households reported seeing a rat in the house in the past month, 860 (97.5%) reported seeing cockroaches in the house in the past month, 677 (76.8%) had a tile or concrete rather than dirt floor, 546 (61.9%) had a trash bin in the home (of which 153 covered the bin), 353 (40.0%) had electricity, and 155 (17.6%) had a drinking water source within 50 meters of the home.

In total, 675 (76.5%) of the 882 households reported at least one case of malaria (as defined by the household) in the month prior to the survey, with a total of 910 (16.8%) of the 5410 individuals reported to have had malaria during that time period. However, 540 (59.3%) of these 910 individuals were presumptively diagnosed by the ill person or a household member, and only 370 (40.7%) were diagnosed following laboratory testing.

A total of 1402 (25.9%) of the individuals within participating households were reported to have had any type of febrile illness (whether caused by malaria or another condition) within the past six months. The rate of fever reported differed by age group and by sex, with young children (those 0 to 4 years old) having the highest rate ($p<0.001$) and females reporting more fevers than males ($p<0.001$) (Table 1). Only 33.9% of people with fever were reported to have had laboratory tests to determine the cause of the fever. There were significant differences in the likelihood of testing by age ($p=0.011$). Households with indicators of higher socioeconomic status (SES), such as those with electricity in the home or a drinking water source very near to the home, generally reported slightly lower rates of febrile illness within the household (Table 2). Markers of household SES were not significantly associated with reported testing rates.

Self-diagnosis of several other conditions was common (Table 3). All of the 234 individuals reported to have ever had influenza, as defined by the household, indicated a self-diagnosis. More than 96% of the 160 persons reported to have had what the household considered to be yellow fever (which had been the focus of a recent vaccination campaign [11]) reported self-diagnosis, as did more than 60% of the 445 persons reported to have had typhoid (which is a relatively common laboratory diagnosis in Bo, as per Widal tests). More than half of the 317 people reported to have had what the household designated as pneumonia were self-diagnosed. However, diagnosis of less common and more specific conditions, such as bacterial meningitis, hepatitis B, and hepatitis C, were nearly always reported to have been diagnosed by a doctor and not self-diagnosed by the household.

Discussion

We found that the majority of febrile illnesses in Bo, Southern Province, Sierra Leone, are self-diagnosed without clinical examination or laboratory testing, including the more than half of suspected malaria cases that are treated presumptively without any clinical diagnostics. The fact that households with greater numbers of individuals were more likely to report that at least one household member had been tested for a febrile illness supports the validity of reports about diagnosis and testing rates, since a greater number of persons in the household increases the probability of a severe illness occurring for at least one resident. The validity of the survey instrument is also supported by the low numbers of households reporting uncommon diseases such as bacterial meningitis unless these conditions were diagnosed by a doctor.

These results are similar to those from studies in other parts of West Africa which have found that more than half of adults self-diagnose fevers and self-medicate for what they consider to be malaria [12,13]. Of those who self-treat, only a small proportion know the correct dosage for common antimalarial medications [12].

This high rate of self-treatment is concerning, since it is likely that a significant proportion of these presumptive cases are treated inappropriately (as per the introduction to this paper). Some people who would benefit from antibiotic and supportive therapy may not be receiving adequate care, and many people who purchase medication for their self-diagnosed malaria or other conditions may be taking drugs that are ineffective for their condition. Febrile individuals who self-treat may experience disease complications and increased treatment costs resulting from delayed access to appropriate medications and other therapy [14]. Taking the wrong medication, such as taking antimalarials for a fever caused by a different infectious agent, generally means that the actual infection remains untreated for at least several days.

When tests are available at a reasonable cost, testing before treating may significantly reduce the inappropriate use of antimicrobial medications. For example, a cohort study of children ages 1 to 10 years in Uganda found that only 32% of fevers were caused by malaria, and the researchers concluded that a test- before-treating approach in that study population would reduce the use of antiparasitic drugs by two-thirds [15]. The reduction of unnecessary treatment achieved by a test-then-treat approach will, by definition, be lower in higher-endemicity areas, including parts of Sierra Leone, where a recent community-based study in the Bo area found that 83% of febrile women and young children who were tested had malaria [16]. Even so, testing may significantly reduce the number of people taking unnecessary medications. The 17% of the 17,130 tested individuals who were malaria-negative in the Bo

area study represented nearly 3000 people who avoided the unnecessary use of antimalarial drugs [16].

Testing does not have to occur in a clinical setting. In rural Sierra Leone, free malaria testing by community health workers (CHWs) has increased access to diagnosis for thousands of households [16]. Those who test positive for malaria are treated by the CHWs, and those with negative malaria tests or complicated malaria cases are referred to a nearby hospital for advanced care (although the follow-up rate for referrals from this program has been shown to be very low). In some places, testing and treatment by CHWs has been found to be preferred over home treatment [17]. However, one potential challenge to testing programs is convincing healthcare providers and patients not to prescribe or take antimicrobials after a negative test result. A study from Ghana found that more than half of patients who tested negative for malaria were prescribed antimalarials anyway [18], and some studies from other parts of Africa have found similar results [19]. Taking antimalarial medication "just in case" may be seen as the best option when households do not have the resources to travel to and pay for further clinical examination and testing.

Besides the direct benefits to patients, expanded use of testing before treatment may slow the further development of drug resistance by reducing the proportion of the population accessing pharmaceutical agents without prescriptions tailored to their actual diagnoses. Patients who have seen a clinician and have a confirmed diagnosis may be more likely than others to complete a full course of an appropriate antibiotic or antimalarial, especially if their treating clinicians counsel them about the importance of compliance with prescribed treatments (an occurrence dependent on those practitioners having the time and resources to provide health education). Community-based behavior change communication processes may also help to promote healthy use of pharmaceuticals by households and communities, but when these public health structures are not in place the burden of health education typically falls on clinicians.

Limitations. This study had several limitations that require a conservative interpretation of the findings. No laboratory tests were conducted to confirm the reported causative agents for participants' febrile illnesses, so we do not know how often their self-diagnoses were accurate. Additionally, participants were not asked about their use of antibiotic and antiparasitic drugs, such as what medications they preferred to take when febrile or where they procured these medications. Because these questions were not part of the survey, we have limited information about whether the self-treatment used by febrile participants was appropriate for their illnesses.

Conclusion

This study provides evidence that self-diagnosis and self-medication for malaria and other febrile illnesses is common in Bo, Sierra Leone. In order to better understand the implications of self- diagnosis and presumptive treatment on patient health outcomes, we recommend that further studies evaluate the types of infections common in this population to see how well clinical laboratory results match self-diagnoses. Future research should also examine the pharmaceutical access and use habits of local residents to see whether appropriate courses of medication are being taken by those with and without prescriptions from a clinician. Understanding the knowledge, attitudes and beliefs, and health practices and behaviors of residents regarding diagnosis and treatment of fevers may contribute to improved health services, policies, and practices.

Competing interests

The authors declare that they have no competing interests.

Disclaimer

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Authors' contributions

RA, KHJ, AAG, MHH, TAL, APM, BL, MJB, and DAS were involved in the conception and design of the study. RA and JML collected the data. RA and KHJ conducted data analysis and drafted the manuscript. All authors critically reviewed the manuscript and approved its submission.

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Tables

Table 1: Prevalence of reported febrile illnesses and testing in the past 6 months, by age and sex

Table 2: Household environmental characteristics and reported febrile illnesses in the household (HH)

Table 3: Reports of family medical history, by source of diagnosis

References

1. Diap G, Amuasi J, Boakye I, Sevcsik AM, Pecoul B. Anti-malarial market and policy surveys in sub-Saharan Africa. Malaria J. 2010; 9(suppl 1):S1. PubMed | Google Scholar

2. Gerstl S, Dunkley S, Mukhtar A, Baker S, Maikere J. Successful introduction of artesunate combination therapy is not enough to fight malaria: results from an adherence study in Sierra Leone. Trans R Soc Trop Med Hyg. 2010; 104(5):328-335. PubMed | Google Scholar

3. Checchi F, Roddy P, Kamara S, et al. Sierra Leone Antimalarial Efficacy Study Collaboration. Evidence basis for antimalarial policy change in Sierra Leone: five in vivo efficacy studies of chloroquine, sulphadoxine-pyrimethamine and amodiaquine. Trop Med Int Health. 2005; 10(2):146-153. PubMed | Google Scholar

4. Guerin PJ, Brasher C, Baron E, et al. Case management of a multidrug-resistant Shigella dysenteriae serotype 1 outbreak in

a crisis context in Sierra Leone, 1999-2000. Trans R Soc Trop Med Hyg. 2004; 98:635-643. PubMed | Google Scholar

5. Sahr F, Solayida AA, Hanson C, Kanty TV, George TA, Harding D. Nasal carriage of Staphylococcus aureus and antibiotic susceptibility pattern among children in Freetown, Sierra Leone. Sierra Leone J Biomedical Res. 2010; 2(1):65-69. PubMed | Google Scholar

6. Zignol M, Hosseini MS, Wright A, et al. Global incidence of multidrug-resistant tuberculosis. J Infect Dis. 2006; 194(4):479-485. PubMed | Google Scholar

7. Nnedu ON, Rimel B, Terry C, Jalloh-Vos H, Baryon B, Bausch DG. Syndromic diagnosis of malaria in rural Sierra Leone and proposed additions to the National Integrated Management of Childhood Illness Guidelines for Fever. Am J Trop Med Hyg. 2010; 82(4):525-528. PubMed | Google Scholar

8. Tayler-Smith K, Kociejowski A, de Lamotte N, et al. Free treatment, rapid malaria diagnostic tests and malaria village workers can hasten progress toward achieving the malaria related Millennium Development goals: the Médecins Sans Frontières experience from Chad, Sierra Leone and Mali. J Public Health Africa. 2011; 2:e12. PubMed | Google Scholar

9. Hopkins H, Asiimwe C, Bell D. Access to antimalarial therapy: accurate diagnosis is essential to achieving long term goals. BMJ. 2009; 339:b2606. PubMed | Google Scholar

10. Ansumana R, Malanoski AP, Bockarie AS, et al. Enabling methods for community health mapping in developing countries. Int J Health Geogr. 2010; 9:56. PubMed | Google Scholar

11. Pezzoli L, Conteh I, Kamara W, et al. Intervene before leaving: clustered lot quality assurance sampling to monitor vaccination coverage at health district level before the end of a yellow fever and measles vaccination campaign in Sierra Leone in 2009. BMC Public Health. 2012; 12:415. PubMed | Google Scholar

12. Nsagha DS, Njunda AL, Kamga HL, et al. Knowledge and practices relating to malaria in a semi-urban area of Cameroon: choices and sources of antimalarials, self-treatment and resistance. Pan African Med J. 2011; 9:8. PubMed | Google Scholar

13. Uzochukwu BSC, Onwujekwe OE. Socio-economic differences and health seeking behavior for the diagnosis and treatment of malaria: a case study of four local government areas operating the Bamako initiative programme in south-east Nigeria. Int J Equity Health. 2004; 364(9448):1896-1898. PubMed | Google Scholar

14. Amexo M, Tolhurst R, Barnish G, Bates I. Malaria misdiagnosis: effects on the poor and vulnerable. Lancet. 2004; 364:1896-1898. PubMed | Google Scholar

15. Njama-Meya D, Clark TD, Nzarubara B, Staedke S, Kamya MR, Dorsey G. Treatment of malaria restricted to laboratory- confirmed cases: a prospective cohort study in Ugandan children. Malaria J. 2007; 6:7. PubMed | Google Scholar

16. Thomson A, Khogali M, de Smet M, et al. Low referral completion of rapid diagnostic test-negative patients in community-based treatment of malaria in Sierra Leone. Malaria J. 2011; 10:94. PubMed | Google Scholar

17. Onwujekwe O, Dike N, Ojukwu J, et al. Consumers stated and revealed preferences for community health workers and other strategies for the provision of timely and appropriate treatment of malaria in southeast Nigeria. Malaria J . 2006; 5:117. PubMed | Google Scholar

18. Ansah EK, Narh-Bana S, Epokor M, et al. Rapid testing for malaria in settings where microscopy is available and peripheral clinics where only presumptive treatment is available: a randomized controlled trial in Ghana. BMJ. 2010 Mar 5;340:c930. PubMed | Google Scholar

19. Graz B, Willcox M, Szeless T, Rougemont A. ?Test and treat? or presumptive treatment for malaria in high transmission situations? A reflection on the latest WHO guidelines. Malaria J. 2011; 10:136. PubMed | Google Scholar

Table 1: Prevalence of reported febrile illnesses and testing in the past 6 months, by age and sex

Age Group	Total			Females			Males			p-value for Chi-squared test of difference by sex (2-tailed)	
	n	n (%) reported to have had fever	Of those reported to have had a fever, n (%) who had tests to determine the cause of the fever	n	n (%) reported to have had fever	Of those reported to have had fever, n (%) tested	n	n (%) reported to have had fever	Of those reported to have had fever, n (%) tested	reported fever cases	testing for cause among those reported to have had fever
0-4	705	368 (52.2%)	136 (37.0%)	348	193 (55.5%)	72 (37.3%)	357	175 (49.0%)	64 (36.6%)	0.088	0.885
5-14	1527	423 (27.7%)	128 (30.3%)	835	233 (27.9%)	77 (33.0%)	692	190 (27.5%)	51 (26.8%)	0.847	0.169
15-29	1739	293 (16.8%)	99 (33.8%)	973	181 (18.6%)	53 (29.3%)	766	112 (14.6%)	46 (41.1%)	0.027	0.040
30-44	803	136 (16.9%)	58 (42.6%)	423	86 (20.3%)	30 (34.9%)	380	50 (13.2%)	28 (56.0%)	0.003	0.018
45-59	381	95 (24.9%)	36 (37.9%)	180	56 (31.1%)	19 (33.9%)	201	39 (19.4%)	17 (43.6%)	0.009	0.351
≥60	255	87 (34.1%)	19 (21.8%)	134	52 (38.8%)	12 (23.1%)	121	35 (28.9%)	7 (20.0%)	0.090	0.749
Total	5410	1402 (25.9%)	476 (34.0%)	2893	801 (27.7%)	263 (32.8%)	2517	601 (23.9%)	213 (35.4%)	0.001	0.309

1402 (25.9%) of household members reported fever, of whom 476 (34.0%) were tested to determine the cause of the fever. In total, 801 (27.7%) females and 601 (23.9%) males reported fever, and 263 (32.8%) of females and 213 (35.4%) of males with fever were tested. The rate of fevers reported was different by sex and by age group, but there were not significant age and sex differences in testing.

Table 2: Household environmental characteristics and reported febrile illnesses in the household (HH)

Household (HH) feature (among 882 households)	1+ HH member reported to have had malaria in the past month			Of those HHs with malaria, 1+ person with malaria reported to have had malaria testing			1+ HH member reported to have had any febrile illness in the past 6 months			Of those HHs reporting fever, 1+ person with fever reported to have had a formal diagnosis		
	among HHs with this feature	among HHs without this feature	p-value	among HHs with this feature	among HHs without this feature	p-value	among HHs with this feature	among HHs without this feature	p-value	among HHs with this feature	among HHs without this feature	p-value
Fewer than six individuals in the household (n=552, 62.8% of HHs)	417 (75.3%)	258 (78.7%)	0.252	125 (30.0%)	102 (39.5%)	0.011	453 (81.8%)	269 (82.0%)	0.932	129 (28.5%)	172 (63.9%)	<0.001
Households with at least one bednet (n=691, 78.3%)	517 (74.8%)	158 (82.7%)	0.020	176 (34.0%)	51 (32.3%)	0.686	584 (84.5%)	138 (72.3%)	<0.001	248 (42.5%)	53 (38.4%)	0.387
Having a tile or concrete floor rather than a dirt floor (n=677, 76.8%)	508 (75.0%)	167 (81.5%)	0.055	177 (34.8%)	46 (27.5%)	0.081	539 (79.6%)	183 (89.3%)	0.001	217 (40.3%)	84 (45.9%)	0.183
Having a trash bin in the home (n=546, 61.9%)	388 (71.1%)	287 (85.4%)	<0.001	138 (35.6%)	85 (29.6%)	0.105	428 (78.4%)	294 (87.5%)	0.001	163 (38.1%)	138 (46.9%)	0.018
Having electricity (n=353, 40.0%)	264 (74.8%)	411 (77.7%)	0.320	90 (34.1%)	133 (32.4%)	0.641	271 (76.8%)	451 (85.3%)	0.002	112 (41.3%)	189 (41.9%)	0.880
Using a drinking water source within 50 meters of the home (n=155, 17.6%)	106 (68.4%)	569 (78.3%)	0.010	27 (25.5%)	196 (34.4%)	0.070	124 (80.0%)	598 (82.3%)	0.505	52 (41.9%)	249 (41.6%)	0.949

Malaria and febrile illnesses were significantly more likely to be reported by households without at least one bednet, those with a dirt floor, those without a trash bin in the home, those without electricity, and those without a drinking water source near the home. Testing of at least one household member was less likely to be reported by smaller households and those without a trash bin in the home.

Table 3: Reports of family medical history, by source of diagnosis			
Condition	n (%) of households reporting that someone currently living in the household has ever had this condition	Of households reporting this condition, n (%) reporting that the condition was diagnosed by a doctor	Of households reporting this condition, n (%) reporting that the condition was self-diagnosed
Influenza	234 (26.5%)	0 (0%)	234 (100%)
Lassa fever	5 (0.6%)	0 (0.0%)	5 (100%)
Yellow fever	160 (18.1%)	6 (3.8%)	154 (96.3%)
Common cold	774 (87.8%)	180 (23.3%)	594 (76.7%)
Asthma	79 (9.0%)	21 (26.6%)	58 (73.4%)
Typhoid fever	445 (50.5%)	177 (39.8%)	268 (60.2%)
Pneumonia	317 (35.9%)	151 (47.6%)	166 (52.4%)
Bacterial meningitis	10 (1.1%)	8 (80.0%)	2 (20.0%)
Hepatitis B	5 (0.6%)	5 (100%)	0 (0.0%)
Hepatitis C	1 (0.1%)	1 (100%)	0 (0.0%)
Tuberculosis	0 (0.0%)	0 (0.0%)	0 (0.0%)
Rift Valley fever	0 (0.0%)	0 (0.0%)	0 (0.0%)
Dengue fever	0 (0.0%)	0 (0.0%)	0 (0.0%)
Households reported a high rate of self-diagnosis for conditions such as influenza (100% of reported cases self-diagnosed), yellow fever (96.3% self-diagnosed), colds (76.7% self-diagnosed), typhoid fever (60.2% self-diagnosed), and pneumonia (52.4% self-diagnosed).			

Reemergence of Chikungunya Virus in Bo, Sierra Leone

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We diagnosed 400 possible IgM-positive cases of chikungunya virus in Bo, Sierra Leone, during July 2012–January 2013 by using lateral flow immunoassays. Cases detected likely represent only a small fraction of total cases. Further laboratory testing is required to confirm this outbreak and characterize the virus.

Outbreaks of infection with chikungunya virus (CHIKV), an alphavirus that is transmitted by bites of infected *Aedes* spp. mosquitoes, were frequent in sub-Saharan Africa and southern and Southeast Asia during the 1950s–1970s, but the infection largely disappeared in the 1980s; only sporadic cases were observed (*1*). The virus reemerged in the early 2000s; major outbreaks were reported in Kenya, some island nations in the Indian Ocean, and several countries in Asia (*2*,*3*).

The primary symptoms of CHIKV infection are high fever (>38.5°C [102°F]) and severe pain in the distal joints of the extremities or the lumbar spine. A maculopapular rash, sensorineural impairment, severe headache, and other nonspecific symptoms may also occur. Symptoms usually resolve within 1–2 weeks after onset of fever, but for a sizeable proportion of patients, arthralgia and arthritis become chronic and pain persists for years (*2*,*3*).

A nationwide serosurvey in Sierra Leone in 1972 detected cases of CHIKV infection throughout the country (*4*), but we are not aware of any cases reported since the mid-1970s. Two recent developments made reemergence appear imminent. First, outbreaks of reemerging CHIKV have been reported in neighboring Guinea (*5*) and in Senegal (*6*). Second, recent yellow fever cases in Sierra Leone have shown that *Aedes* spp. mosquito–borne infections are common (*7*). Thus, it was not surprising when we initiated an infectious disease surveillance study in July 2012 in the city of Bo, in Southern Province, Sierra Leone, that we detected possible chikungunya virus infections. We report initial results of our investigation.

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The Study

On July 7, 2012, the Mercy Hospital Research Laboratory (MHRL) in Bo, Sierra Leone, initiated a 1-year infectious disease surveillance program to identify the diversity of pathogens causing febrile illness in the city. A tiered analysis approach was used. First, all specimens from febrile study participants were tested for H12 infections with various pathogens, including CHIKV, by commercially available test kits. Specimens that showed negative results in this first round of testing were further tested by using cultures, multiplex PCR, and resequencing pathogen microarrays. The research protocol was approved by Njala University, George Mason University, the Liverpool School of Tropical Medicine, the US Naval Research Laboratory, and the Sierra Leone Ethics and Scientific Review Committee.

During July 7, 2012–January 10, 2013, MHRL conducted first-tier lateral flow immunoassay (LFI) tests of blood samples from all 932 outpatients >5 years of age who had been clinically examined at the hospital, were found to have febrile illness, and consented to having blood drawn for laboratory testing. LFI test kits (SD Bioline; Standard Diagnostics, Inc., Seoul, South Korea) were used for diagnosis of IgM against CHIKV; IgG and IgM against dengue virus and hepatitis A virus; hepatitis B virus surface antigen, hepatitis C virus, HIV-1/2, and antibodies against these viruses; and IgG and IgM against *Leptospira* spp., *Salmonella enterica* Serovar typhi, and syphilis.

Most patients reported that they had sought medical care within several days after the onset of their febrile illnesses. Levels of IgM against CHIKV are usually detectable by immunochromatographic methods within a few days after infection and persist for H3–4 months (*1*,*2*). The LFI test kits for CHIKV were reported by the manufacturer to have a sensitivity of 97.1% and a specificity of 91.1% compared with those of ELISA (*8*). An independent evaluation found a sensitivity of 50.8% and a specificity of 89.2% for the kits; sensitivity ranged from 40.9% 1–5 days after onset of illness to 65.4% 16–20 days after onset (*9*). Specificity decreases after the first week (*10*).

More than half of the cases tested during the first week of the surveillance program were positive by LFI for CHIKV. Thus, we notified the Sierra Leone Ministry of Health and Sanitation of a possible CHIKV outbreak. By January 10, 2013, 400 (42.9%) of 932 febrile patients were positive by LFI for CHIKV (Figure 1). Ages of the 400 CHIKV IgM-positive patients ranged from 6 years to

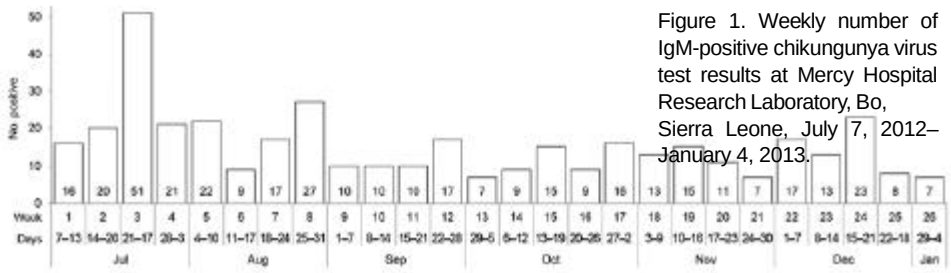

Figure 1. Weekly number of IgM-positive chikungunya virus test results at Mercy Hospital Research Laboratory, Bo, Sierra Leone, July 7, 2012–January 4, 2013.

85 years; 172 (43.0%) were male patients. Of these 400 patients, 220 (55.0%) reported arthralgia, 189 (47.3%) chills, and 156 (39.0%) headaches. Co-infections were common; 92 (23.0%.) were co-infected with malaria, 37 (9.3%) with HIV, 33 (8.3%) with hepatitis B virus, and smaller numbers with hepatitis A, hepatitis C, tuberculosis, typhoid, and syphilis. Four CHIKV-positive samples were also positive for dengue.

On July 28, MHRL launched an Ushahidi-based website (www.ushahidi.com) to compile case reports. Details about the patients who were positive for CHIKV were uploaded to the MHRL website (www.mhrlsl.com/GIA/ushahidi) and, if possible, were geolocated on an open street map (www.openstreetmap.org) that linked to a map of Bo created by MHRL for health research purposes (*11*). The map showed that the cases were located throughout Bo (Figure 2). Of the 400 LFI-positive case-patients, 319 (79.8%) could be mapped; the remainder did not provide a home street address on the laboratory patient information form. However, the sample was not population-based because Mercy Hospital is 1 of several hospitals serving Bo, so a city-wide attack rate could not be determined.

Results of attempts by the US Naval Research Laboratory to confirm the LFI results by using semi-nested reverse transcription PCR on fast technique for analysis of nucleic acid–preserved samples were inconclusive, possibly because of genetic sequence variation from well-characterized strains or because of the timing of specimen collections. Viral loads for humans with CHIKV infection decrease after the second day of symptoms, and viral titers may be low after the fifth day (*12*,*13*). Because CHIKV nucleic acids are only detectable in serum for a few days, reverse transcription PCR results are often discordant with those of serologic (IgM and IgG) assays. Confirmation that

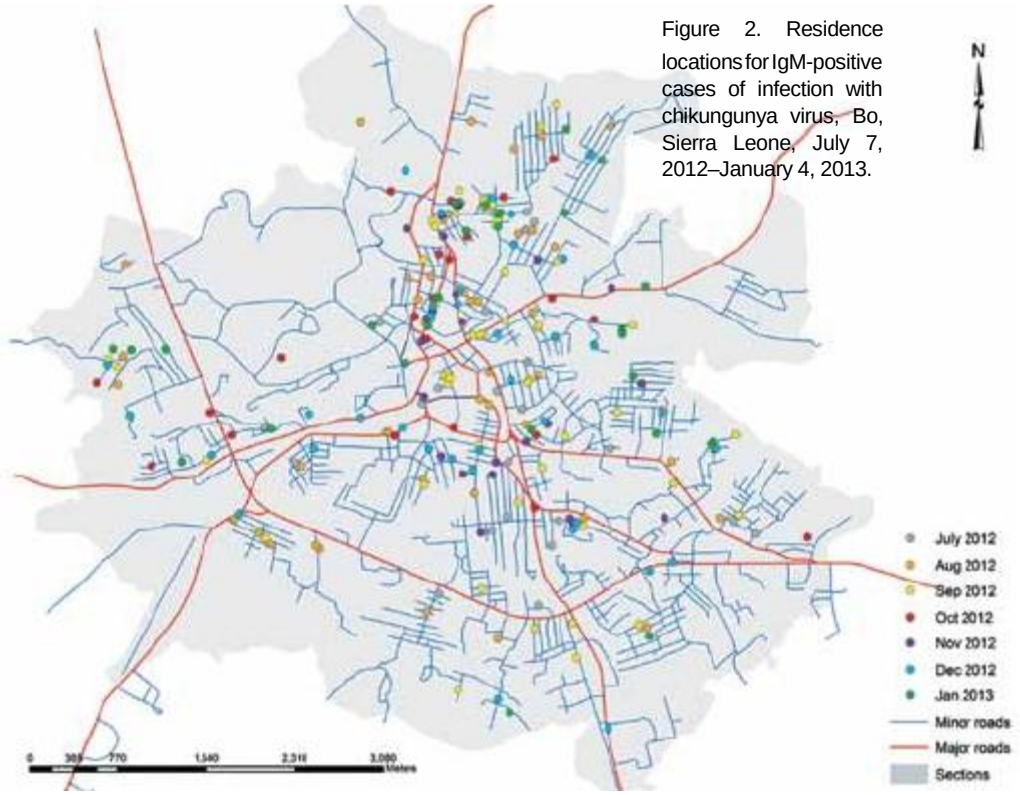

Figure 2. Residence locations for IgM-positive cases of infection with chikungunya virus, Bo, Sierra Leone, July 7, 2012–January 4, 2013.

an outbreak occurred is dependent on isolation of the virus, followed by molecular characterization, full-genome sequencing, and phylogenetic mapping.

Conclusions

Our results suggest that an outbreak of chikungunya virus occurred in Sierra Leone. The exact time of the reemergence of this virus cannot be pinpointed, but retrospective analyses of outpatient charts suggested that, on the basis of syndromic criteria, the first cases occurred in January 2012 and the outbreak peaked during the rainy season in 2012. Other outbreaks reported in central and west Africa have also occurred during the rainy season, which is typical for *Aedes* spp. mosquito–borne infections (*6*,*14*,*15*). Because Mercy Hospital serves only a relatively small proportion of the residents of Bo, the cases detected likely represent only a small fraction of the total cases that have occurred. Further study will be required to confirm the laboratory results and, if further investigation is warranted, to document the extent of the outbreak.

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References

1. Ng LC, Hapuarachchi HC. Tracing the path of chikungunya virus: evolution and adaptation. Infect Genet Evol. 2010;10:876–85. ttp://dx.doi.org/10.1016/j.meegid.2010.07.012
2. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise RT. Chikungunya: a re-emerging virus. Lancet. 2012;379:662–71. http://dx.doi.org/10.1016/S0140-6736(11)60281-X
3. Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clin Infect Dis. 2009;49:942–8. http://dx.doi.org/10.1086/605496
4. Mouchet J, Robin Y. Serological and entomological survey on yellow fever in Sierra Leone: report on a mission May 16–June 7, 1972. Report no. AFR/YF/18. Geneva: World Health Organization; 1973.
5. Jentes ES, Robinson J, Johnson BW, Conde I, Sakouvougui Y, Iverson J, et al. Acute arbovirual infections in Guinea, west Africa, 2006. Am J Trop Med Hyg. 2010;83:388–94. http://dx.doi.org/10.4269/ajtmh.2010.09-0688
6. Thonnon J, Spiegel A, Diallo M, Diallo A, Fontenille D. Epidémies à virus chikungunya en 1996 et 1997 au Sénégal. Bull Soc Pathol Exot. 1999;92:79–82.
7. World Health Organization. Outbreak news: yellow fever, Sierra Leone. Wkly Epidemiol Rec. 2011;86:101–2.
8. Standard Diagnostics, Inc. Chikungunya IgM [cited 2012 Sep 12]. http://www.standardia.com/html_e/mn03/mn03_01_00.asp?intId=119
9. Kosasih H, Widjaja S, Surya E, Hadiwijaya SH, Butarbutar DP, Jaya UA, et al. Evaluation of two IgM rapid immunochromatographic tests during circulation of Asian lineage chikungunya virus. Southeast Asian J Trop Med Public Health. 2012;43:55–61.
10. Rianthavorn P, Wuttirattanakowit N, Prianantathavorn K, Limpaphayom N, Theamboonlers A, Poovorawan Y. Evaluation of a rapid assay for detection of IgM antibodies to chikungunya. Southeast Asian J Trop Med Public Health. 2010;41:92–6.
11. Ansumana R, Malanoski AP, Bockarie AS, Sundufu AJ, Jimmy DH, Bangura U, et al. Enabling methods for community health mapping in developing countries. Int J Health Geogr. 2010;9:56. http://dx.doi.org/10.1186/1476-072X-9-56
12. Laurent P, Le Roux K, Grivard P, Bertil G, Naze F, Picard M, et al. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify chikungunya virus. Clin Chem. 2007;53:1408–14. http://dx.doi.org/10.1373/clinchem.2007.086595
13. Ray P, Ratagiri VH, Kabra SK, Lodha R, Sharma S, Sharma BS, et al. Chikungunya infection in India: results of a prospective hospital based multi-centric study. PLoS ONE. 2012;7:e30025. http://dx.doi.org/10.1371/journal.pone.0030025
14. Caron M, Paupy C, Grard G, Becquart P, Mombo I, Nso BB, et al. Recent introduction and rapid dissemination of chikungunya virus and dengue virus serotype 2 associated with human and mosquito co-infections in Gabon, Central Africa. Clin Infect Dis. 2012;55:e45–53. http://dx.doi.org/10.1093/cid/cis530
15. Demanou M, Antonio-Nkondjio C, Ngapana E, Rousset D, Paupy C, Manuguerra JC, et al. Chikungunya outbreak in a rural area of western Cameroon in 2006: a retrospective serological and entomological survey. BMC Res Notes. 2010;3:128. http://dx.doi.org/10.1186/1756-0500-3-128

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Lymphedema in a 7-year-old boy infected with Wuchereria bancrofti in Sierra Leone: A case report

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abstract

We present a case of congenital lymphedema in a 7-year-old boy in Sierra Leone with active filarial infection and penile edema. The genital edema with onset at 6 months of age may have been due to a congenital abnormality in lymphatic drainage. Other possible causes of childhood lymphedema, including Milroy's disease, are discussed.

1. Introduction

Lymphatic filariasis (LF) is a major cause of acute and chronic morbidity manifested as lymphedema and hydrocele. The clinical signs and symptoms of LF affect more than 40 million people globally, making the disease the second-largest cause of permanent and long-term disability worldwide (Shenoy and Bockarie, 2011; Chu et al., 2010). LF is a major obstacle to socioeconomic development in the 73 countries across Africa, Southeast Asia, the Americas, and the Pacific region that are known to be endemic for the mosquito-borne disease (WHO, 2013; Addiss, 2010).

LF infection is often acquired in childhood in areas of high transmission intensity, but infected individuals usually do not develop endemic areas up to 10% of children under the age of 15 years are infected (Bockarie et al., 2002). In 2000, the World Health Organization launched the Global Program to Eliminate LF (GPELF) using the pro-poor strategy of preventive chemotherapy and transmission control (PCT) together with vector control, morbidity management, and disability prevention (WHO, 2013). The mass drug administration (MDA) strategy used for PCT combines albendazole with either ivermectin or diethylcarbamazine citrate (DEC). However, not all at-risk children in high-risk areas are able to access MDA.

Lymphatic filariasis caused by Wuchereria bancrofti is highly endemic in Sierra Leone, where more than 90% of the 6.6 million people are at risk of acquiring the infection. (Hodges et al., 2012; Koroma et al., 2012) Filarial disease is common in all the endemic districts in the country, but there is no record previously of

2. Case report

During a transmission assessment survey to determine the success of four rounds of MDA on reducing LF transmission in north

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Fig. 1. Lymphedema of Right Lower Extremity.

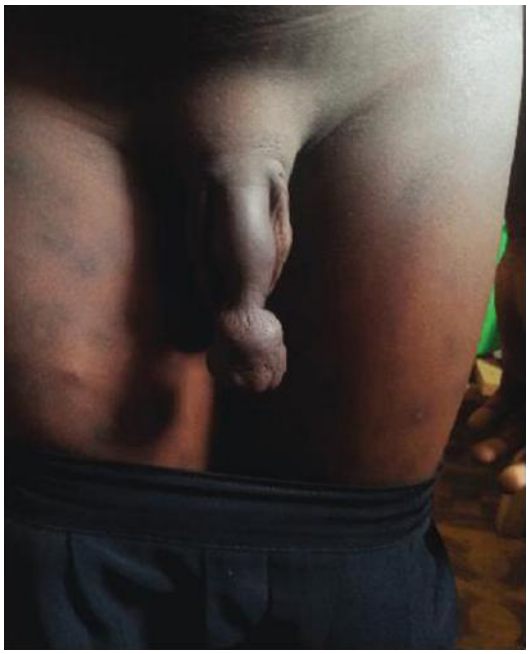

Fig. 2. Deformed Prepuce.

central Pujehun District, Sierra Leone, a 7-year-old boy from a rural village presented with chronic progressive lymphedema of the right lower extremity. The overlying tissue of the distal extremity was indurated, possibly due to secondary infection and tissue trauma, and there were dermatologic changes of the right foot consistent with elephantiasis (Fig. 1). The temperature of the swollen areas was normal, with no signs of nodular or warty excrescences. The boy's father reported that asymmetrical non-pitting edema had been present since birth, and had increased in severity over time. Keratosis spots were present over the entire body, and the prepuce of the penis showed a significant deformity that the father said had been present since the child was six months of age (Fig. 2). The father gave permission for photographs of the child and case information to be published.

The boy tested positive for circulating filarial antigenemia using a BinaxNOW filariasis Immuno Chromatographic Test (ICT) (Inverness Medical Professional Diagnostics, Preehold, NJ, USA), and a night blood smear examination with Giemsa stain confirmed Bancroftian filariasis with 313 W. bancrofti mf/ml of blood prior to treatment. The parasite was sheathed with nuclei in the tail end and was morphologically distinguishable from the Mansonella species which can also be found in human blood in Africa. These observations and the species-specific ICT positive result confirmed that the microfilaria observed in the blood were W. bancrofti. Initial treatment prescribed for the active filarial infection was a 6 mg dose of ivermectin and a 400 mg dose of albendazole every three months with follow-up. The father reported that the boy had not been previously treated for LF infection.

The boy had lived with his parents in rural Pujehun District for the past four years, and they previously resided in Kono District (Fig. 3). Before her pregnancy with the boy and during the end of her first trimester, his mother had resided in Kono District with her grandmother, a resident of Bombali District, who had elephantiasis. Both Bombali and Kono Districts were endemic for LF prior to the MDA and the birth of the boy (Koroma et al., 2013).

As part of the investigation of the child's disease, family members were examined for LF. The mother tested LF positive based on both ICT and night blood smear examination, and reported taking only one round of MDA during her lifetime. The boy's father and the boy's only sibling both tested negative. None of the immediate family members had anatomical abnormalities related to LF. However, a family history suggested that the boy's deceased maternal grandmother likely also had an advanced filarial infection, as she had progressive lymphedema of the lower right extremity that developed over the last five years of her life.

Two months after the boy's initial diagnosis, following prescribed treatment with ivermectin and albendazole, a night blood smear test showed no evidence of circulating microfilaria. A complete blood count, comprehensive metabolic panel, and urinalysis were all found to be within normal limits. A physical examination revealed no evidence of another inciting condition. Both the child and his father were also tested at this time for several other infectious diseases, including human immunodeficiency virus (HIV), dengue, leptospirosis, chikungunya, malaria, syphilis, onchocerciasis, and hepatitis A, B, and C. Both tested positive for malaria, with all other tests negative.

The patient was prescribed a daily dose of 200 mg of doxycycline, which has been shown to cause significant reductions in lymphedema in patients with Grade II–III LF following 12–24 months of treatment, regardless of circulating filarial status (Mand et al., 2012). Although some risk of adverse effects has been reported in administration of doxycycline to children under 10 years old, the treating physician concluded that with appropriate safety monitoring the benefits of doxycycline for this patient outweighed the potential risks. Additionally, the ballooning foreskin of the boy was removed through circumcision. The biopsied tissue was unremarkable.

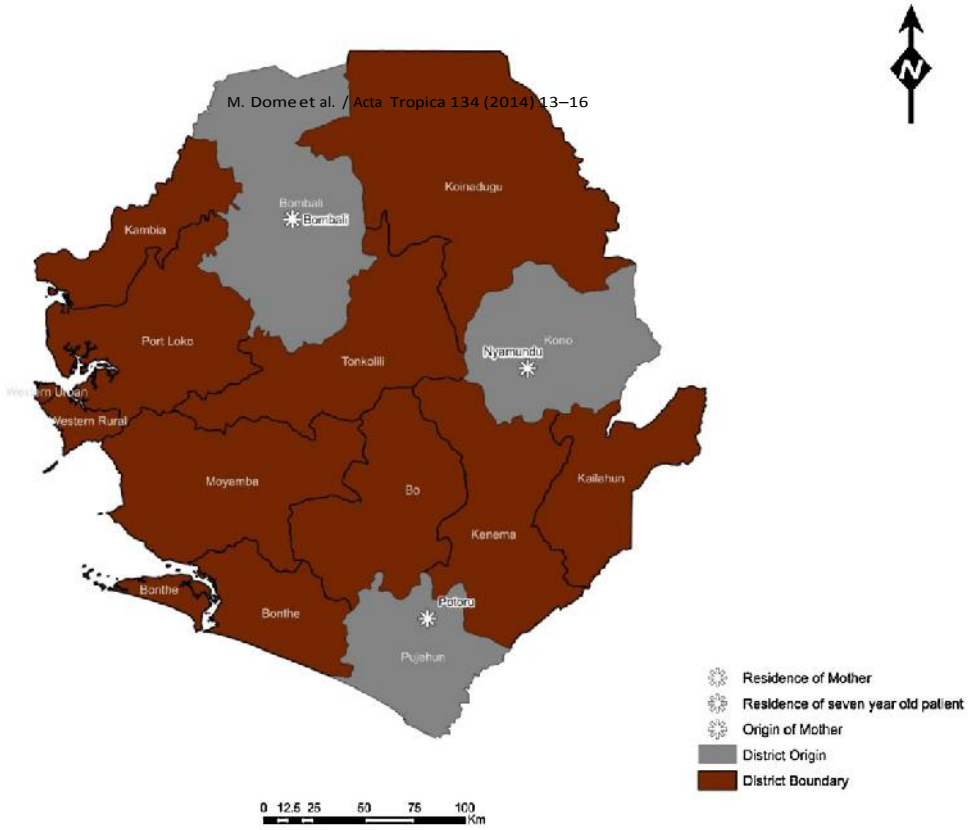

Fig. 3. Map of Sierra Leone Showing Location of Case Patient and Familial Relations.

3. Discussion

Lymphatic filariasis is rarely symptomatic in children, especially those who are less than 10 years old, and when children do have symptoms LF rarely presents as lymphedema (Bockarie et al., 2009). A comprehensive meta-analysis of LF in children by Witt and Ottesen (2001) identified only 5 cases of lymphedema or elephantiasis of a limb in children less than 10 years old in the published literature, most of which occurred in the mid-20th century. A study from the mid-1990s of 441 Indian children (ages 1–14 years) with LF found only 12 cases of lymphedema (Harinath et al., 2000). Thus, the case patient appears to have an unusual presentation of lower limb enlargement in a 7-year old boy infected with W. bancrofti.

One possible cause of this congenital deformity is Milroy's disease, an autosomal dominant genetic disorder characterized by lymphedema, especially lymphedema of the legs, that is present at birth or develops in infancy (Makhoul et al., 2002). A 2011 case report from an LF non-endemic area described a healthy full-term newborn with no complications during pregnancy or delivery who developed swelling of his right leg soon after birth (Perez-Crespo et al., 2011). As observed in our case, the lower limb swelling was more pronounced in the distal part of the leg and foot. The skin showed non-pitting edema and no swelling signs in the area. The family history was also similar to ours. The grandmother and some maternal relatives were also found to be affected with lymphedema, including the child's grandmother. Based on the family history and clinical examination alone, the patient was diagnosed with Milroy's disease. Similar cases described elsewhere were also clinically diagnosed as Milroy's disease (Connell et al., 2008; Kitsiou-Tzeli et al., 2010). Milroy's disease had also been associ- ated with swelling of the genitals, as per a case report from Gueglio et al. (2003) of giant scrotal lymphedema due to Milroy's disease. No relative of the 7-year old boy was known to have been born with lymphedema, but up to 10% of persons with Milroy's disease may be asymptomatic (Brice et al., 2005). Advanced molecular diagnostics would be required to confirm that the lymphedema in the 7-year old observed in Sierra Leone was due to Milroy's disease.

This study suggests that lymphedema due to LF in children may be difficult to diagnose because of the rarity of symptomatic LF in the pediatric population. In LF-endemic areas, the possibility of other causes of lymphedema in infected children should be care-

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References

Addiss, D., 2010. The 6th Meeting of the Global Alliance to Eliminate Lymphatic Filariasis: A half-time review of lymphatic filariasis elimination and its integration with the control of other neglected tropical diseases. Parasit. Vectors 3, 100.

Bockarie, M.J., Taylor, M.J., Gyapong, J.O., 2009. Current practices in the management of lymphatic filariasis. Expert Rev. Anti Infect. Ther. 7, 595–605.

Bockarie, M.J., Tisch, D.J., Kastens, W., Alexander, N.D.E., Dimber, Z., Bockarie, F., Ibam, E., Alpers, M.P., Kazura, J.W., 2002. Mass treatment to eliminate filariasis in Papua New Guinea. N Engl J Med. 347, 1841–1848.

Brice, G., Child, A.H., Evans, A., Bell, R., Mansour, S., Burnand, K., Sarfarazi, M., Jeffery, S., Mortimer, P., 2005. Milroy disease and the VEGFR-3 mutation phenotype. J. Med. Genet. 42, 98–102.

Chu, B.K., Hooper, P.J., Bradley, M.H., McFarland, D.A., Ottesen, E.A., 2010. The economic benefits resulting from the first 8 years of the Global Programme to Eliminate Lymphatic Filariasis (2000–2007). PLoS Negl. Trop. Dis. 4, e708.

Connell, F., Brice, G., Mortimer, P., 2008. Phenotypic characterization of primary lymphedema. Ann. N.Y. Acad. Sci. 1131, 140–146.

Gueglio, G., Quijada, E., Salas, H., Daels, P., Tejerizo, J., Chernobilsky, V., Giúdice, C., Damia, O., 2003. Giant scrotal lymphedema caused by Milroy's disease. Arch Esp Urol. 56 (8), 949–952.

Harinath, B.C., Reddy, M.V.R., Bhandari, B.B., Mehta, V.K., Chaturvedi, P., Prajapati, N.C., Gupta, R.K.C., 2000. Filaria associated clinical manifestations in children in an endemic area and morbidity control by immunomonitoring and optimal DEC therapy: Sevagram experience. Indian J. Clin. Biochem. 15 (Suppl. 1), 118–126.

Hodges, M.H., Sonnie, M., Turay, H., Conteh, A., MacCarthy, F., Sesay, S., 2012. Maintaining effective mass drug administration for lymphatic filariasis through in-process monitoring in Sierra Leone. Parasit. Vectors 5, 232.

Koroma, J.B., Sesay, S., Sonnie, M., Hodges, M.H., Sahr, F., Zhang, Y., Bockarie, M.J., 2013. Impact of three rounds of mass drug administration on lymphatic filariasis in areas previously treated for onchocerciasis in Sierra Leone. PLoS Negl. Trop. Dis. 7, e2273.

Mand, S., Debrah, A.Y., Klarmann, U., Batsa, L., Marfo-Debrekyei, Y., Kwarteng, A., Specht, S., Belda-Domene, A., Fimmers, R., Taylor, M., Adjei, O., Hoerauf, A., 2012. Doxycycline improves filarial lymphedema independent of active filarial infection: a randomized controlled trial. Clin. Infect. Dis. 55, 621–630.

Makhoul, I.R., Sujov, P., Ghanem, N., Bronshtein, M., 2002. Prenatal diagnosis of Milroy's primary congenital lymphedema. Prenat. Diagn. 22, 823–826.

Perez-Crespo, M., Betlloch, I., Martinez-Miravete, M.T., Ballester, I., Lucas, A., Mataix, J., 2011. Congenital lower limb enlargement in a newborn. Pediatr. Dermatol. 28, 579–580.

Sequence Variability and Geographic Distribution of Lassa Virus, Sierra Leone

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Lassa virus (LASV) is endemic to parts of West Africa and causes highly fatal hemorrhagic fever. The multimammate rat (*Mastomys natalensis*) is the only known reservoir of LASV. Most human infections result from zoonotic transmission. The very diverse LASV genome has 4 major lineages associated with different geographic locations. We used reverse transcription PCR and resequencing microarrays to detect LASV in 41 of 214 samples from rodents captured at 8 locations in Sierra Leone. Phylogenetic analysis of partial sequences of nucleoprotein (NP), glycoprotein precursor (GPC), and polymerase (L) genes showed 5 separate clades within lineage IV of LASV in this country. The sequence diversity was higher than previously observed; mean diversity was 7.01% for nucleoprotein gene at the nucleotide level. These results may have major implications for designing diagnostic tests and therapeutic agents for LASV infections in Sierra Leone.

Lassa fever (LF) belongs to a group of viral hemorrhagic fevers characterized by a febrile case-fatality rates (*1*). LF differs from most viral hemor-rhagic fevers in that it is endemic to a large geographic area of sub-Saharan Africa. Human cases of LF have been reported in (or imported from) Guinea, Sierra Leone, Liberia, Mali, Burkina Faso, and Nigeria; however, LF outbreaks seem to be restricted to Guinea, Sierra Leone, Liberia (the Mano River Union region), and Nigeria (*2*–*4*). In some ar- eas of Sierra Leone and Guinea, more than half of the pop- ulation has antibodies against Lassa virus (LASV; family *Arenaviridae*), the etiologic agent of LF (*5*,*6*). According to various estimates, 300,000–500,000 cases of LF result in 5,000–10,000 deaths annually in West Africa (*6*,*7*). An analysis based on seroepidemiologic data suggested that the number of cases might be much higher, reaching 3 million cases and 67,000 fatalities per year (*8*). Overall, the population at risk might include as many as 200 million persons living in a large swath of West Africa from Senegal to Nigeria and beyond (*4*).

LASV can cause infection in the multimammate rat (*Mastomys natalensis*), a natural host and reservoir of this pathogen (*9*,*10*). The multimammate rat is a commensal rodent ubiquitous in Africa (*11*,*12*). Although the routes of LASV infection are poorly characterized, humans probably get infected by eating contaminated food (*13*), by inhaling virus-contaminated aerosols (*14*), or while butchering infected rat meat (*15*). Person-to-person transmission of LASV is well documented, mostly in the form of nosoco- mial outbreaks (*13*).

Like other arenaviruses, LASV is an enveloped virus with a bisegmented single-stranded RNA genome encod- ing 4 proteins using an ambisense coding strategy (*16*). The small segment contains genes for the glycoprotein precursor (GPC) and nucleoprotein (NP), which serves as the main viral capsid protein. The large segment encodes the small zinc-binding protein (Z), which contains a RING motif, and another gene (L) containing the RNA-dependent RNA polymerase domain.

Complete genome sequences are available for several LASV strains, as are a considerable number of partial sequences from isolates originating from humans and rodents (*17*–*20*). Their analysis revealed the existence of high sequence diversity (up to 27% nt) and 4 major lineages of LASV, which correlate with geographic location (*17*). Lineages I, II, and III, and the greatest diversity of LASV strains, were found among isolates from Nigeria, whereas strains from Guinea, Sierra Leone, and Liberia seemed to be more closely related and belong exclusively to lineage IV. Sequence of the AV strain (*21*) and recently published sequences from rodent LASV isolates from Mali (*18*)

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[1]These authors contributed equally to this article.

suggest the existence of an additional clade (proposed as lineage V) (*22*). LASV sequences of isolates from humans and rodents are found interspersed throughout the phyloge- netic tree, which is consistent with the notion that human cases typically result from transmission from rodents (*17*).

The high degree of sequence divergence of LASV genomes is a major problem affecting the development of molecular and immune-based diagnostic technologies, vaccines, and possibly antiviral drugs (*13*,*16*,*17*,*23*–*25*). Forty-seven unique partial LASV sequences from Si- erra Leone were available in GenBank at the time of this analysis, which included fragments of NP (27 sequences), GPC (9 sequences), L (9 sequences), and Z (2 sequences) genes plus full sequences of small and large segments of 2 strains—Josiah and NL. Most of these sequences are from isolates collected >30 years ago; only 2 more recent se- quences (GPC and L gene fragments) from strain SL06-2057 were isolated in 2006 (*17*,*19*).

To fill this gap, we investigated the sequence diversity of strains circulating among small rodents captured in peri- domestic settings in Sierra Leone. In 2014, we screened 214 samples collected during 2009 from several species of rodents trapped in villages where LF was reported in hu- mans. We used diagnostic reverse transcription PCR (RT-PCR) and high-density resequencing microarrays to detect LASV and amplify fragments of NP, GPC, and L genes. The obtained amplicons were sequenced and compared with

Methods

Rodent Sample Collection

The rodent samples collected were part of a separate project (L.M. Moses, unpub. data). Thirteen locations were select- ed for study in the LF-endemic region of eastern Sierra Le- one. The geographic coordinates of the sampling locations and details of rodent trapping methods are available in the online Technical Appendix (Technical Appendix Table 1, Rodent Trapping Procedures, http://wwwnc.cdc.gov/EID/ article/21/4/14-1469-Techapp1.pdf). Traps with captured small animals were processed in remote areas outside of the villages according to approved guidelines (*26*). The animals were anesthetized with isoflurane, and their morpho- metrics recorded. Animals were euthanized by exsangui- nation using cardiac puncture or cervical dislocation, and necropsies were performed. Spleen sections were stored in RNALater or TRIzol for RNA extraction (Life Technolo- gies, Grand Island, NY, USA). Rodents were identified to the genus level in the field. Animals identified as *Mastomys* sp. were further identified down to species level by using molecular methods as described previously (*27*).

Nucleic Acid Extraction

RNA from 10 mg of spleen of each rodent was extracted with TRIzol following the manufacturer's recommenda- tions. The samples were stored at –80°C.

RT-PCR and Sequencing

RNA were reverse-transcribed by using the SuperScript III Reverse Transcriptase kit (Life Technologies) according to the manufacturer's instructions, and RT products were stored at –20°C. Specific oligonucleotide primer pairs were used for the PCR targets of interest (Table 1) at final con- centrations of 0.25 μM each. For PCR, 2 μL of RT reaction was used as template in 25 μL reactions containing 1.25 mM dNTPs, 1× Taq buffer, 0.2 μM each of primers, and 1.25 U FastStart Taq enzyme (Roche Diagnostics, India- napolis, IN, USA). NP targets were amplified by using an initial 2-min denaturation at 95°C, followed by 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. Some specimens produced poor PCR products, with low yields or multiple bands when we used published primer pair 1010C/ OW1696R (*17*); 1 μL of PCR product from those speci- mens was amplified in nested PCR by using the primer pair LAS_NP_F_1/LAS_NP_R_1 and the same thermal cycling program to generate DNA fragments suitable for sequencing (Table 1). GPC targets were amplified by us- ing 36E2 and LVS339-rev primers (*24*) and a PCR profile consisting of 2-min denaturation at 95°C, followed by 45 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min. L gene targets were amplified by using modified prim- ers, LVL3359-F and LVL3754-R, based on published se- quences (*28*) and a PCR program consisting of 2-min dena- turation at 95°C followed by 45 cycles of 95°C for 30 sec,

Table 1. PCR and sequencing primers used in study of Lassa virus, Sierra Leone*

Primer name	Sequence, 5′ → 3′	Target gene	Amplicon size	Reference
1010C	TCIGGIGAIGGITGGCC	NP	670	(*17*)
OW1696R	AIATGAIGCAGTCCAIIAGTGCACAGTG			(17)
LAS_NP_F_1	GGGTGGCCATAYATTGCATC		650	
LAS_NP_R_1	GTCCAGGAGTGCACAGTGAG			This study
36E2	ACCGGGGATCCTAGGCATTT	GPC	317	(*24*)
LVS339-rev	GTTCTTTGTGCAGGAMAGGGGCATKGTCAT			
LVL3359-F	AGAATYAGTGAAAGGGARAGCAATTC	L	394	(*28*)
LVL3754-R	CACATCATTGGTCCCCATTTACTRTGATC			

*GPC, glycoprotein precursor; L, polymerase; NP, nucleoprotein.

53°C for 30 sec, and 72°C for 1 min. PCR amplicons were size-confirmed by electrophoresis by using 1.2% FlashGel DNA cassettes (Lonza, Walkersville, MD, USA) and purified on Zymo DNA Clean & Concentrator columns (Zymo Research, Irvine, CA, USA). All DNA sequencing was performed by Eurofins MWG Operon (Huntsville, AL, USA). The sequences were deposited into GenBank under the following accession numbers: NP sequences, KM406518–KM406556; GPC sequences, KM406590–KM406623; and L sequences, KM406557–KM406589.

RPM-TEI Microarray Analysis

The resequencing pathogen microarray (RPM) analysis was conducted by using Tropical and Emerging Infections microarrays (RPM-TEI v. 1.0; TessArae, Potomac Falls, VA, USA). The RPM-TEI microarray enables detection of84 biothreat agents, including all lineages of LASV (*29*). Sample preparation was conducted as previously described (*29*). Pathogen identification was performed using the "C3Score" identification algorithm (*30*).

Phylogenetic Analysis

We conducted the sequence alignment using the MUSCLE algorithm implemented in the MEGA 6.0 software package (*31*). In addition to partial NP, GPC, and L sequences obtained in this study, we included in the alignments all homologous sequences from these genes in samples collected in Sierra Leone (or clustering with Sierra Leone sequences) available in GenBank. Twenty-seven NP, 10 GPC, and 8 L sequences were available that meet these criteria. To root the trees, sequences from more distantly related, lineage IV isolate Z-158, which originated from Macenta district in Guinea, were used as an outgroup on the basis of the previ- ous phylogenetic analyses (*17*). We also used MEGA 6.0 to perform statistical selection of the nucleotide substitution model for each sequence collection. We selected the Tamu- ra 3-parameter model with discrete γ-distributed rate varia- tion as the best-fitting model for NP and L sequence sets and the Kimura 2-parameter model with a fraction of evo- lutionary invariant sites for GPC sequences. The phylog- enies were inferred by using the Bayesian, Markov Chain Monte Carlo method, as implemented in MrBayes v3.2.2 (*32*). The analysis was run without an assumption of a mo- lecular clock. The resulting phylogenies were presented as 50% majority rule consensus trees in which the branches with posterior probability <0.5 were collapsed into polyto- mies. We manually adjusted the trees using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree).

Results and Discussion

We collected 681 small mammals during the field survey. Of these, we analyzed 214 for this study on the basis of RNA availability at the time of the study (online Technical Appendix Table 2). These samples were obtained from rodents captured at 13 locations in 3 districts within the southern and eastern provinces of Sierra Leone (Figure 1; online Technical Appendix Table 1). The rodents belonged to 6 genera; 199 were identified as *M. natalensis*, which is consistent with published data on the ubiquitous presence of this species in domestic environments in West Africa (*11*,*12*). The other rodents (identified to genus level only) were *Rattus* sp. (9 [4.2%] rodents); *Cricetomys* sp. (3 [1.4%] rodents); and *Mus* sp., *Praomys* sp., and *Hylomyscus* sp. (1 [0.5%] rodent each).

We screened all samples for LASV by RT-PCR using pan–Old World arenavirus (OWA) primers (*17*), which amplify a 670-nt section of NP gene. Because of poor results of NP amplification using OWA primers (inefficient amplification, multiple bands), we modified the screening protocol to include a second nested PCR using primers internal to the OWA amplification product, designed to amplify 650-nt segment of NP sequence and be more specific for lineage IV NP gene sequences. We obtained sequencing-quality NP amplicons from 39 samples using this protocol. For all of these NP-positive samples, we attempted to obtain RT-PCR amplicons for fragments of GPC and L genes using previously published or modified primers (*17*,*24*,*28*). The GPC and L amplifications were successful for most NP-positive samples and failed only in 5 and 6 samples, respectively (Table 2).

In addition, we screened a randomly selected subset of 51 samples (representing 8 collection sites and 4 rodent genera; online Technical Appendix Table 2) for nucleic acids of 84 different pathogens using RPM-TEI v. 1.0. Of the 51 samples tested using RPM-TEI, 9 were positive for LASV, and no other pathogens were detected in the analyzed samples. Although the percentage of positive samples was similar to that from RT-PCR results, the RPM-TEI failed to detect LASV in 3 samples that were positive for the NP gene by 2-step RT-PCR (LM34, LM58, and LM68). All these samples originated from the same village (Bumpeh), and no sample from this site was RPM-TEI positive, which suggests that detection failures might have been caused by inefficient amplification of the sequence variant of LASV circulating in the Bumpeh area with the RPM-TEI primers. On the other hand, RPM-TEI detected viral RNA in 2 RT-PCR–negative samples (LM591 and LM649), bringing to41 the number of samples positive for LASV nucleic acids(Table 2).

In summary, 41 LASV-positive samples were obtained from animals captured in 8 locations: Barlie (13 samples), Largo (8 samples), Bumpeh (7 samples), Ngiehun (4 sam- ples), Koi and Yawei (3 samples each), Taiama (2 sam- ples), and Saama (1 sample) (Figure 1). No LASV RNA was detected in samples collected from Gouma, Joru, Ken- ema, Panguma, or Segbwema. Lack of LASV detection in

Table 2. Results of Lassa virus detection among rodent samples with >1 positive test result, Sierra Leone*

Sample	Date collected	Collection site		PCR†				
		Village/town	District	NP	GPC	L	RPM‡	
LM0034	2009 Jan 27	Bumpeh	Kenema	+	+	+	Neg	
LM0036	2009 Jan 27	Bumpeh	Kenema	+	+	+	NT	
LM0047	2009 Jan 28	Bumpeh	Kenema	+	+	Neg	NT	
LM0054	2009 Jan 28	Bumpeh	Kenema	+	+	+	Neg	
LM0058	2009 Jan 29	Largo	Kenema	+	+	+	NT	
LM0064	2009 Jan 30	Largo	Kenema	+	+	Neg	+	
LM0068	2009 Jan 30	Largo	Kenema	+	+	Neg	NT	
LM0087	2009 Feb 3	Largo	Kenema	+	Neg	+	NT	
LM0091	2009 Feb 3	Largo	Kenema	+	+	+	NT	
LM0092	2009 Feb 3	Koi	Kenema	+	+	+	NT	
LM0093	2009 Feb 3	Koi Koi Ngiehun	Kenema	+	+	+	NT	
LM0111	2009 Feb 4	Ngiehun	Kenema	+	+	+	NT	
LM0122	2009 Feb 5	Ngiehun	Kenema	+	+	+	+	
LM0123	2009 Feb 5	Saama Barlie	Kenema Bo	+	+	+	+	
LM0124	2009 Feb 5	Barlie	Bo	+	+	+	+	
LM0224	2009 Feb 18	Barlie	Bo	+	+	+	NT	
LM0250	2009 Feb 19	Barlie	Bo	+	+	+	NT	
LM0273	2009 Feb 20	Barlie	Bo	+	+	+	+	
LM0395	2009 Jul 22	Barlie	Bo	+	+	Neg	+	
LM0396	2009 Jul 22	Barlie	Bo	+	+	+	NT	
LM0434	2009 Jul 23	Barlie Yawei	Bo	+	+	+	Neg	+
Z0007	2009 Dec 17	Taiama	Kenema	+	+	Neg +	NT	NT

*GPC, glycoprotein precursor; L, polymerase; Neg, negative; NP, nucleoprotein; NT, sample not tested; RPM, resequencing pathogen microarray; +, positive.

Kenema, Panguma, and Segbwema, areas well known to have regular LASV transmission, might be due to the small number of traps used. The town of Joru was extensively trapped, and no LASV was found. This finding is not surprising because Joru is south of the area where LASV is usually found.

All positive samples came from multimammate rats, which is considered the sole vector species for LASV (*13*). The results of LASV detection using several different RT-PCR strategies and a broad-range resequencing microarray (RPM-TEI v. 1.0) showed that none of the techniques applied alone detected viral RNA in all positive samples. This result underscores the difficulty of developing a truly universal diagnostic assay for this highly variable virus, even in the case of closely related strains belonging to lineage IV.

The analysis of the new sequences of LASV strains circulating in rodents in Sierra Leone indicated that the viral genome diversity is higher than previously estimated (*17*). For all available Sierra Leone sequences (including this study) the mean difference calculated for partial NP, GPC, and L sequences was 7.01% nt, 8.92% nt, and 9.83% nt, respectively, and 2.82% aa, 4.06% aa, and 0.71% aa, respectively (Table 3). These differences are higher than the reported 4.6% nt and 1.7% aa differences based on partial NP sequences in a study with fewer isolates (*17*). The L gene fragment seemed to vary the most at the nucleotide level, followed by GPC and NP, which is consistent with previous observations (*33*). However, at the amino acid level, the GPC gene varied most, followed by the NP and L genes. The high conservation of the protein sequence of L

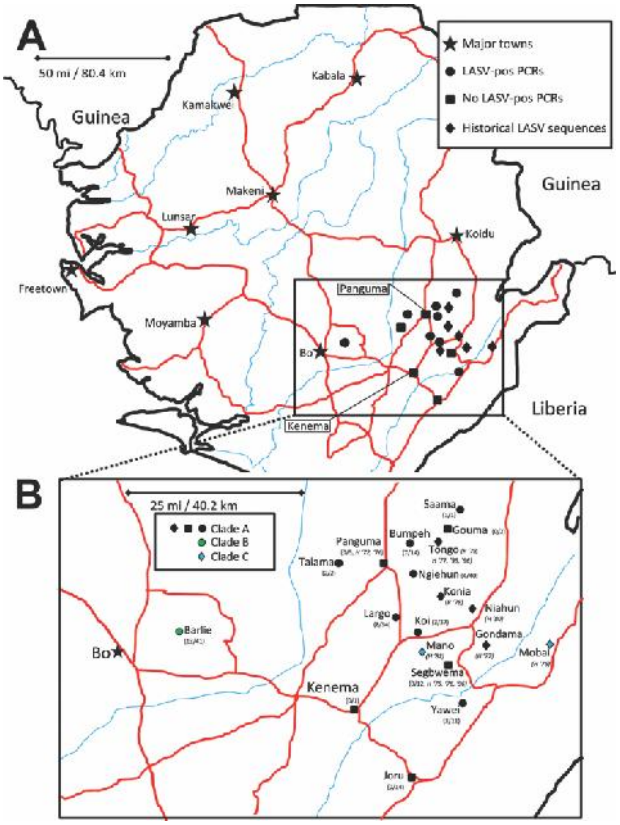

Figure 1. A) Locations of origin for Lassa virus (LASV) nucleic acid sequences, Sierra Leone. B) Enlarged view of region from which rodent specimens were collected. Major roads (red) and waterways (blue) are indicated. Symbols indicate major cities and towns (stars); sites in this study with rodent samples that were PCR positive for LASV (circles); sites in this study from which all samples from mulitmammate rats were PCR negative for LASV (squares); and sites from which published LASV sequences originated (diamonds). The color of the symbols in panel B indicates the clade for nucleoprotein sequence: black, clade A; green, clade B; blue, clade C. Fractions indicate, for each site included in this study, number of PCR-positive samples and total number of samples. Other designations for published sequence sites indicate type of isolate (H, human; R, rodent) and year(s) of isolation. No published information about geographic origin was available for the following strains: 807875, 331, 523, IJ531, Josiah, NL, SL06–2057, SL15, SL20, SL21, SL25, SL26, SL620.

gene fragment analyzed in this study (0.71% mean difference) seemed have resulted from selection of a highly conserved part of L gene (located in RNA polymerase domain) when the diagnostic assay was designed (*28*).

The analysis of phylogenetic trees constructed by using all available partial sequences of NP, GPC, and L genes from Sierra Leone confirmed previous findings that the strains circulating in this country belong to lineage IV and are closely related to each other (*17*,*19*). The topology of the largest NP-based tree (Figure 2) strongly supports the hypothesis that the isolates from Sierra Leone belong to at least 3 distinct major clades (posterior probability 1.00 in all cases): the first clade (A), including a large cluster of strains originating from a group of villages to the north and east of Kenema in the Eastern Province (Bumpeh, Gondama, Koi, Konia, Largo, Ngiehun, Panguma, Segbwema, Taiama, Tongo, and Yawei; Figure 1); the second clade (B), including several strains isolated from rodents cap- tured in Barlie (located a few kilometers southeast of Bo) and 1 isolate from Saama (located northeast of Kenema); and the third clade (C) represented by just 2 older human isolates from Mano and Mobai.

Table 3. Estimates of average evolutionary divergence of NP, GPC, and L gene fragments for Lassa virus strains,

	Difference†	
Gene, grouping	Nucleotide	Amino acid
NP Overall		
Clade A	7.01	2.82
Clade B	5.03	2.06
Clade C	6.44	2.42
GPC		
Overall	8.92	4.06
Clade A	6.26	2.60
Clade B	7.49	3.29
Clade D		
L		
Overall	9.83	0.71
Clade A	6.59	0.58
Clade B	0.88	0.00
Clade D		

*GPC, glycoprotein precursor; L, polymerase; NP, nucleoprotein.

†The numbers of nucleotide and amino acid differences per site from averaging over all sequence pairs or all sequence pairs within a clade multiplied by 100 are shown. All positions containing gaps and missing data were eliminated. Values for clade E defined for GPC and L sequences were not calculated because clade E contained only 1

Phylogenetic trees based on GPC and L sequences (Figures 3, 4) had similar topology and supported existence of clades A (with posterior probabilities 0.74 and 1.00, respectively) and B (with posterior probabilities 1.00 for both trees). However, the clade C was not present because the sequences for GPC and L gene fragments were not available for the strains forming this cluster in the NP-based tree. In addition to clades A and B, GPC- and L-based trees suggested existence of 2 additional and distinct clades. Clade D was represented by 2 sequences from human isolates SL25 and SL26, which formed a separate cluster (posterior probability 1.00 for both trees), and clade E represented by sequences obtained from a single strain isolated in 2006 (SL06–2057). These clades are defined by a very small number of sequences, and the GPC- and L-based trees disagree on the order of their separation from other clades. In addition, no data have been published on geographic origin of clade D and E samples. More data are needed (including corresponding NP sequences) to establish the existence and position of clades D and E with more certainty.

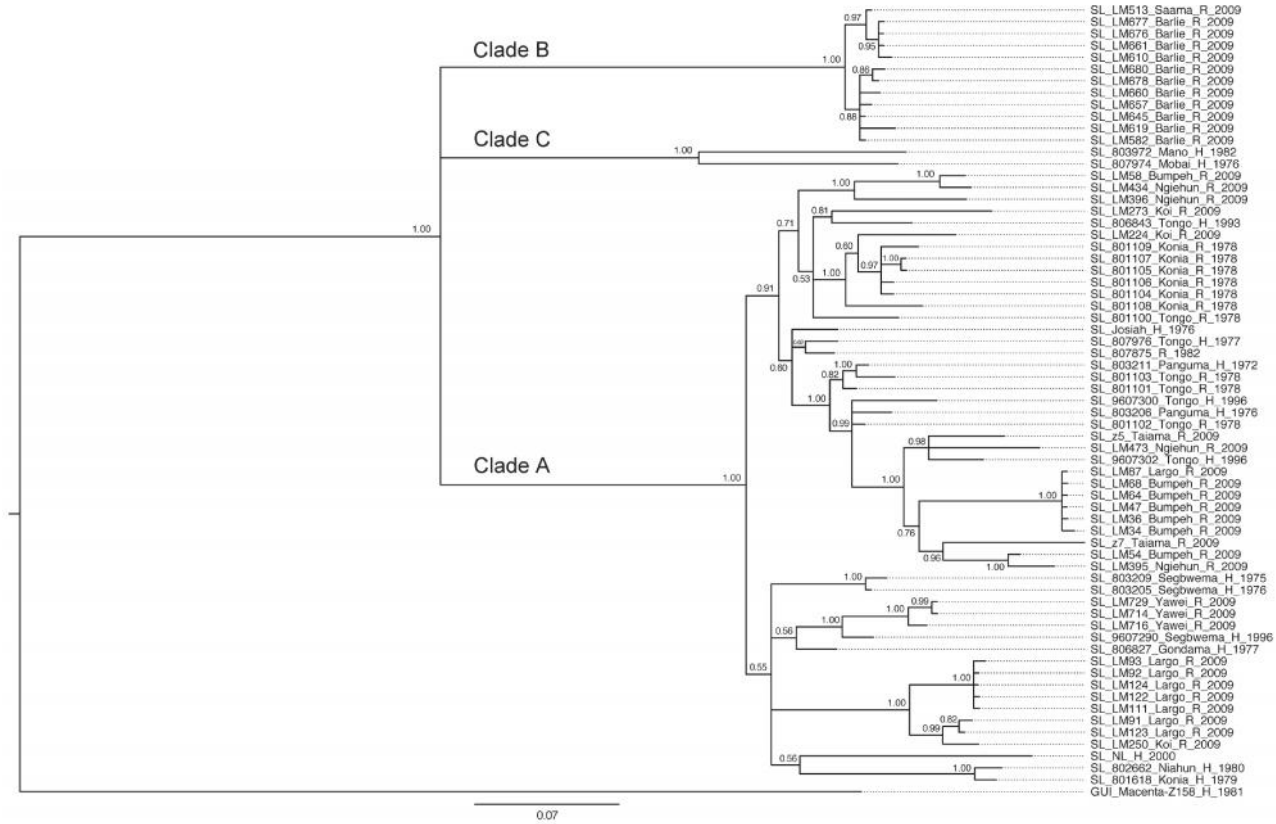

Figure 2. Phylogenetic analysis of Lassa virus isolates from Sierra Leone based on partial nucleoprotein (NP) gene sequences. The homologous NP fragments of 621 nt were aligned. The isolate Z-158, which originated from Macenta district in Guinea, was used as outgroup based on the previous phylogenetic analyses to root the tree. The 50% majority rule consensus tree was estimated by using Bayesian Inference method implemented in MrBayes software (*32*) using the Tamura 3-parameter substitution model with discrete γ-distributed rate variation. The strain labels contain information on the country of origin (SL, Sierra Leone; GUI, Guinea), strain designation, village or town of origin, type of isolate (H, human; R, rodent), and year of isolation. The numbers next to the branches indicate the posterior probability of particular clades. The clades as defined in this study (clades A, B, and C) are also indicated next to the appropriate branches. Scale bar indicates substitutions per site.

All of the trees indicate a high degree of geographic clustering of the strains. This kind of clustering has been reported previously over large geographic distances and is believed to have resulted from limited dispersal and migration of the host species (*17*,*19*). Results of this study show that this phenomenon also can be observed over relatively short distances. Isolates originating from multimammate rat specimens obtained in a particular location tended to cluster, and conversely sequences present in specific branches of the trees in many cases originated from a single location or few locations not far from each other. This kind of clustering could be observed especially well in samples from Barlie, Largo, Bumpeh, Konia, and Yawei (Figures 2–4).

In addition to the general pattern of geographic clustering, in several cases single isolates clustered with strains from different locations. For example, the sequence from a single sample from Saama (LM513) was closely related to that of strains from Barlie (based on NP sequence analysis). In another example, 1 GPC sequence originating from Liberia (523) clustered with Sierra Lone clade A sequences. In some cases (e.g., Saama sample LM513), such unusual clustering patterns may be explained by cross contamination or mislabeling of the samples. They also might result from relative proximity of all sampling sites and inadvertent anthropogenic transfer of rodents. Massive population movements that occurred in Sierra Leone during the 1991–2002 civil war could contribute to the process of mixing multimammate rat subpopulations carrying different LASV strains (*34*).

The geographic location of human cases at such a fine spatial scale can be problematic because humans can move large distances after exposure before disease is detected. For the human isolates, the clustering inconsistent with geographic location might have resulted from recording of the hospital location or patient's current location as strain's origin instead of the actual location of rodent–human trans- mission. For example, the NP-based phylogenetic tree in- dicates that human isolates from Segbwema and Gondama

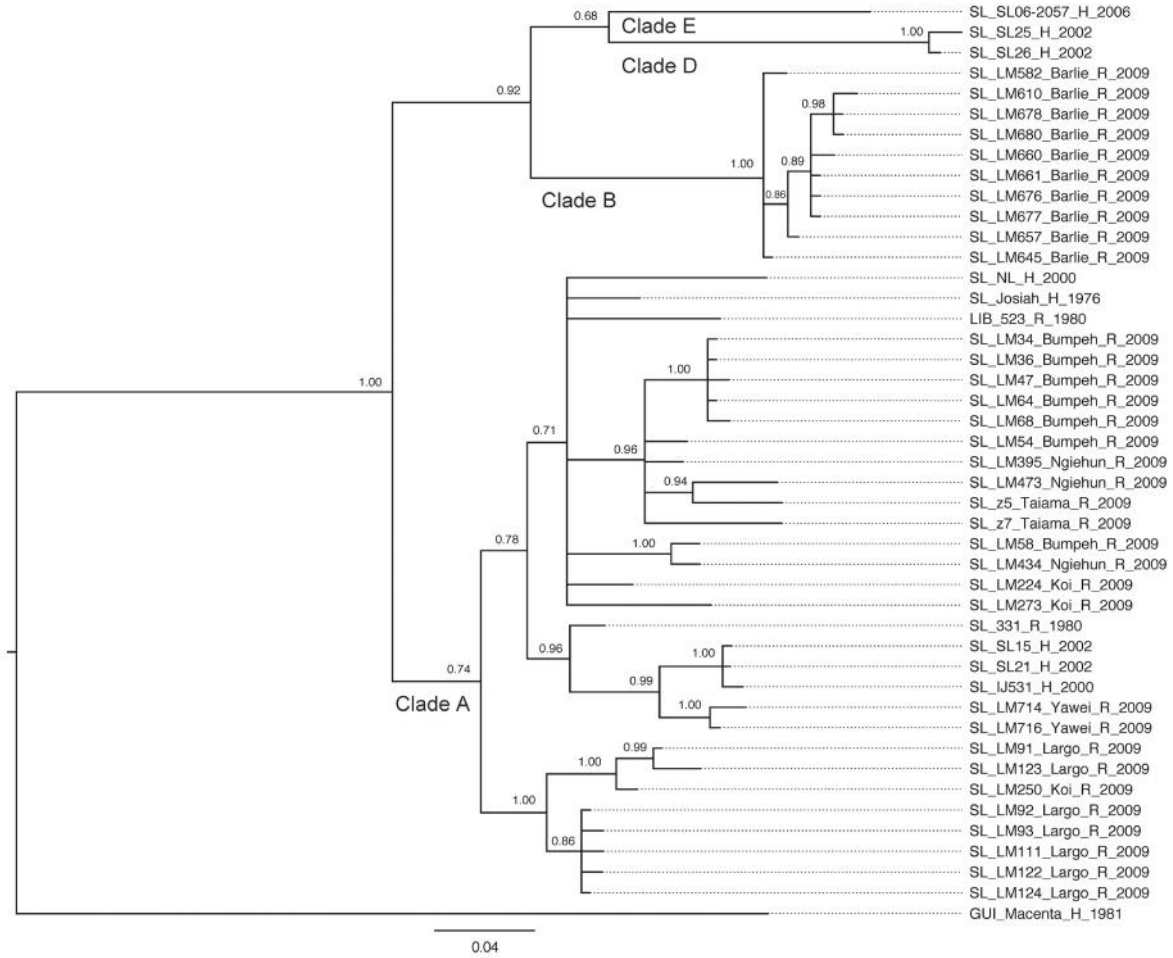

Figure 3. Phylogenetic analysis of Lassa virus (LASV) isolates from Sierra Leone based on partial glycoprotein precursor (GPC) gene sequences. The homologous GPC fragments of 284 nt were aligned. The isolate Z-158, which originated from Macenta district in Guinea were used as outgroup based on the previous phylogenetic analyses to root the tree. The 50% majority rule consensus tree was estimated by using Bayesian Inference method implemented in MrBayes software (*32*) using the Kimura 2-parameter substitution model with a fraction of evolutionary invariant sites. The strain labels contain information on the country of origin (SL, Sierra Leone; GUI, Guinea; LIB, Liberia), strain designation, village or town of origin, type of isolate (H, human; R, rodent), and year of isolation. The numbers next to the branches indicate the posterior probability of particular clades. The clades as defined in this study (clades A, B, D, and E) are also indicated next to the appropriate branches. Scale bar indicates substitutions per site.

(obtained in 1996 and 1977, respectively) most likely originated from the Yawei village area because they cluster closely. A few other human isolates (SL15, SL20, and SL21) for which no location information is available also clustered with Yawei isolates on the basis of GPC and L sequences, suggesting their origin in the same area. These sequences were obtained in 2002 from United Nations peacekeepers stationed in this part of Sierra Leone (*28*,*35*).

Recent epidemiologic data show that LF was detected in 10 of 13 districts in Sierra Leone, which suggests that the infection is much more common that previously recognized (*36*). Phylogenetic analysis of the sequences revealed that strains circulating in districts to the west of the traditional hyperendemic area from which most sequence information is available differ significantly (clade B), which suggests that these could be distinct LASV strains that circulated in local multimammate rat populations for a long time since diverging from a common ancestor and are unlikely to have resulted from recent expansion of this rodent to new areas, as was recently suggested to explain emergence of cases from districts in which LF was not previously reported (*36*). Furthermore, the presence of LASV in Barlie with such high prevalence was surpris- ing because this area historically has had few reports of LASV until 2 human LF cases reported in 2009 (L.M. Moses, unpub. data). The lack of reported LF cases from this area leads to speculation that clade B may be a less pathogenic form of LASV, and transmission to humans might have occurred previously but went unrecognized because of milder, nonhemorrhagic symptoms. In fact, the

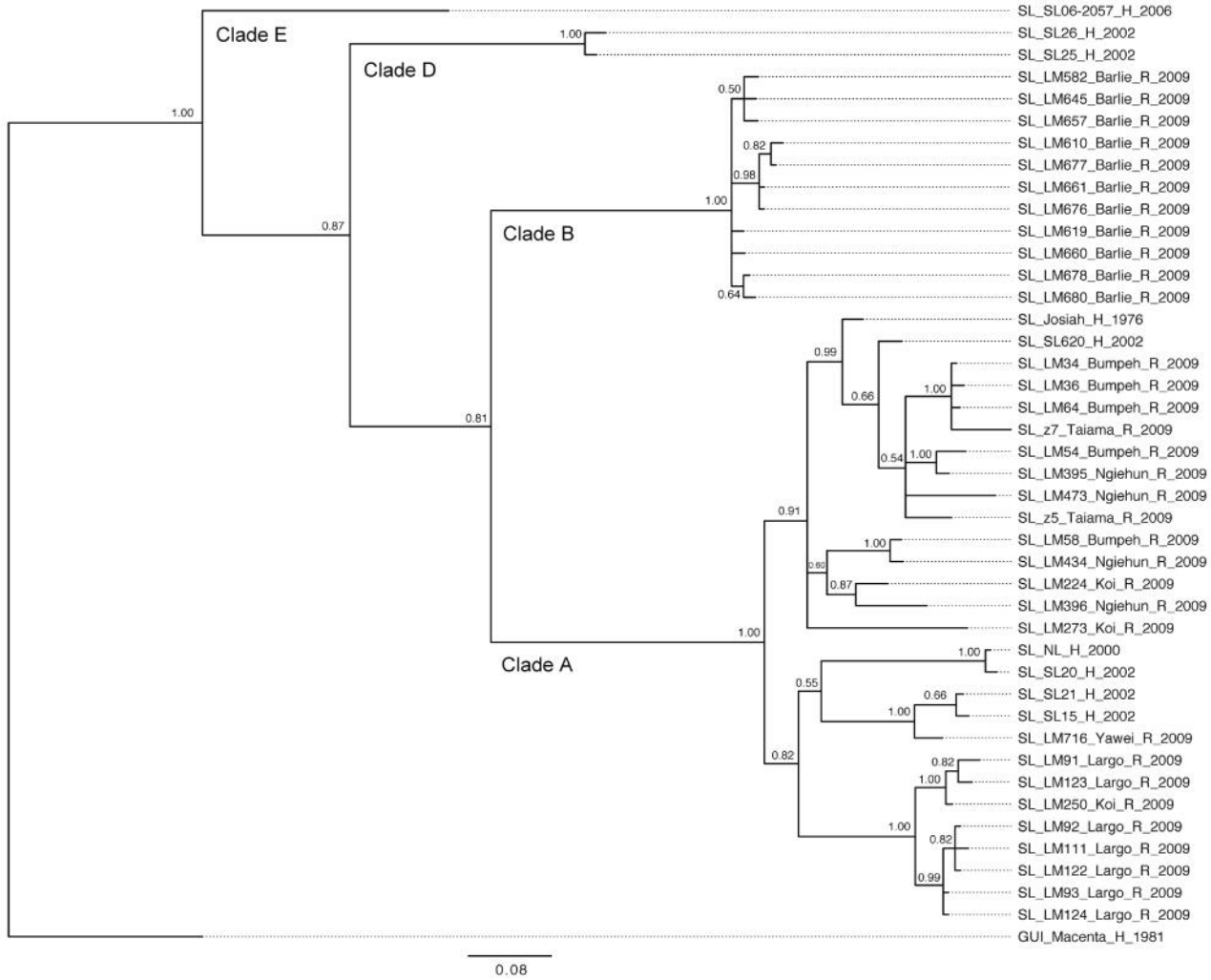

Figure 4. Phylogenetic analysis of Lassa virus (LASV) isolates from Sierra Leone based on partial polymerase (L) gene sequences.

The homologous L fragments of 373 nt were aligned. The isolate Z-158, which originated from Macenta district in Guinea, was used as outgroup based on the previous phylogenetic analyses to root the tree. The 50% majority rule consensus tree was estimated by using Bayesian Inference method implemented in MrBayes software (*32*) using the Tamura 3-parameter substitution model with and a fraction of evolutionary invariant sites. The strain labels contain information on the country of origin (SL, Sierra Leone; GUI, Guinea), strain designation, village or town of origin, type of isolate (H, human; R, rodent), and year of isolation. The numbers next to the branches indicate the posterior probability of particular clades. The clades as defined in this study (clades A, B, D, and E) are also indicated next to the appropriate branches. Scale bar indicates substitutions per site.

idea of broader area of LASV endemicity in Sierra Leone is consistent with results of serosurveys conducted during the 1980s by McCormick, who found seroprevalence levels ranging from 8% in southern coastal areas to 15% in villages in Northern Province (*6*).

Molecular characterization of isolates from a wider geographic area of the country is needed to fully understand the diversity of the LASV strains in Sierra Leone and its impact on disease distribution and risk. Such information would be useful for developing efficient viral detection These diagnostic tests are extremely relevant to disease surveillance and monitoring and evaluation of interventions to prevent primary LASV infection in humans. More extensive information about sequence diversity affecting the antigenicity of the virus or the function of its RNA-dependent RNA polymerase may help in the development of vaccines and antiviral drugs. It will also lead to deeper understanding of the biology and pathogenesis of LASV.

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References

1. Frame JD, Baldwin JM Jr, Gocke DJ, Troup JM. Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings. Am J Trop Med Hyg. 1970;19:670–6.
2. Sogoba N, Feldmann H, Safronetz D. Lassa fever in West Africa: evidence for an expanded region of endemicity. Zoonoses Public Health. 2012;59(Suppl 2):43–7. http://dx.doi.org/10.1111/j.1863-2378.2012.01469.x
3. Macher AM, Wolfe MS. Historical Lassa fever reports and 30-year clinical update. Emerg Infect Dis. 2006;12:835–7. http://dx.doi.org/10.3201/eid1205.050052
4. Fichet-Calvet E, Rogers DJ. Risk maps of Lassa fever in West Africa. PLoS Negl Trop Dis. 2009;3:e388. http://dx.doi.org/10.1371/journal.pntd.0000388
5. Lukashevich IS, Clegg JC, Sidibe K. Lassa virus activity in Guinea: distribution of human antiviral antibody defined using enzyme-linked immunosorbent assay with recombinant antigen. J Med Virol. 1993;40:210–7. http://dx.doi.org/10.1002/jmv.1890400308
6. McCormick JB, Webb PA, Krebs JW, Johnson KM, Smith ES. A prospective study of the epidemiology and ecology of Lassa fever. J Infect Dis. 1987;155:437–44. http://dx.doi.org/10.1093/ infdis/155.3.437
7. McCormick JB. Lassa fever. In: Saluzzo JF, Dodet B, editors. Emergence and control of rodent-borne viral diseases. Amsterdam: Elsevier; 1999. p. 177–95.
8. Richmond JK, Baglole DJ. Lassa fever: epidemiology, clinical features, and social consequences. BMJ. 2003;327:1271–5. http://dx.doi.org/10.1136/bmj.327.7426.1271
9. Monath TP, Newhouse VF, Kemp GE, Setzer HW, Cacciapuoti A. Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone. Science. 1974;185:263–5. http://dx.doi.org/10.1126/science.185.4147.263
10. Walker DH, Wulff H, Lange JV, Murphy FA. Comparative pathology of Lassa virus infection in monkeys, guinea-pigs, and *Mastomys natalensis.* Bull World Health Organ. 1975;52:523–34.
11. Smit A, van der Bank H, Falk T, de Castro A. Biochemical genetic markers to identify two morphologically similar South African *Mastomys* species (*Rodentia*: *Muridae*). Biochem Syst Ecol. 2001;29:21–30. http://dx.doi.org/10.1016/S0305-1978(00)00028-4
12. consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of Guinea. Am J Trop Med Hyg. 1996;55:661–6.
13. Lukashevich I, Salvato MS. Lassa virus genome. Curr Genomics. 2006;7:351–79. http://dx.doi.org/10.2174/138920206778948673
14. Bowen MD, Rollin PE, Ksiazek TG, Hustad HL, Bausch DG, Demby AH, et al. Genetic diversity among Lassa virus strains. J Virol. 2000;74:6992–7004. http://dx.doi.org/10.1128/JVI.74.15.6992-7004.2000
15. Safronetz D, Sogoba N, Lopez JE, Maiga O, Dahlstrom E, Zivcec M, et al. Geographic distribution and genetic characterization of Lassa virus in sub-Saharan Mali. PLoS Negl Trop Dis. 2013;7:e2582. http://dx.doi.org/10.1371/journal.pntd.0002582
16. Ehichioya DU, Hass M, Becker-Ziaja B, Ehimuan J, Asogun DA, Fichet-Calvet E, et al. Current molecular epidemiology of Lassa virus in Nigeria. J Clin Microbiol. 2011;49:1157–61. http://dx.doi.org/10.1128/JCM.01891-10
17. Lecompte E, Fichet-Calvet E, Daffis S, Koulemou K, Sylla O, Kourouma F, et al. *Mastomys natalensis* and Lassa fever, West Africa. Emerg Infect Dis. 2006;12:1971–4. http://dx.doi.org/10.3201/eid1212.060812
18. Günther S, Emmerich P, Laue T, Kuhle O, Asper M, Jung A, et al. Imported Lassa fever in Germany: molecular characterization of a new Lassa virus strain. Emerg Infect Dis. 2000;6:466–76. http://dx.doi.org/10.3201/eid0605.000504
19. Günther S, Weisner B, Roth A, Grewing T, Asper M, Drosten C, et al. Lassa fever encephalopathy: Lassa virus in cerebrospinal fluid but not in serum. J Infect Dis. 2001;184:345–9. http://dx.doi.org/10.1086/322033
20. Trappier SG, Conaty AL, Farrar BB, Auperin DD, McCormick JB, Fisher-Hoch SP. Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection. Am J Trop Med Hyg. 1993;49:214–21.
21. Olschläger S, Lelke M, Emmerich P, Panning M, Drosten C, Hass M, et al. Improved detection of Lassa virus by reverse transcription–PCR targeting the 5′ region of S RNA. J Clin Microbiol. 2010;48:2009–13. http://dx.doi.org/10.1128/JCM.02351-09
22. Demby AH, Chamberlain J, Brown DW, Clegg CS. Early diagnosis of Lassa fever by reverse transcription–PCR. J Clin Microbiol. 1994;32:2898–903.
23. Mills JN, Childs JE, Ksiazek TG, Peters CJ, Wallis MV. Methods for trapping and sampling small mammals for virologic testing. Atlanta: US Department of Health and Human Services; 1995.
24. Lecompte E, Brouat C, Duplantier J-M, Galan M, Granjon L, Loiseau A, et al. Molecular identification of four cryptic species of

33. Vieth S, Torda AE, Asper M, Schmitz H, Gunther S. Sequence analysis of L RNA of Lassa virus. Virology. 2004;318:153–68. http://dx.doi.org/10.1016/j.virol.2003.09.009

34. Lalis A, Leblois R, Lecompte E, Denys C, Ter Meulen J, Wirth T. The impact of human conflict on the genetics of *Mastomys natalen- sis* and Lassa virus in West Africa. PLoS ONE. 2012;7:e37068. http://dx.doi.org/10.1371/journal.pone.0037068

35. UN peacekeepers are at risk. Trop Med Int Health. 2001;6:83–4. http://dx.doi.org/10.1046/j.1365-3156.2001.00676.x

36. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, et al. Lassa fever in post-conflict Sierra Leone. PLoS Negl Trop Dis. 2014;8:e2748. http://dx.doi.org/10.1371/journal.pntd.0002748

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Leapfrog diagnostics: Demonstration of a broad spectrum pathogen identification platform in a resource-limited setting

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Abstract

Background: Resource-limited tropical countries are home to numerous infectious pathogens of both human and zoonotic origin. A capability for early detection to allow rapid outbreak containment and prevent spread to non-endemic regions is severely impaired by inadequate diagnostic laboratory capacity, the absence of a "cold chain" and the lack of highly trained personnel. Building up detection capacity in these countries by direct replication of the systems existing in developed countries is not a feasible approach and instead requires "leapfrogging" to the deployment of the newest diagnostic systems that do not have the infrastructure requirements of systems used in developed countries.

Methods: A laboratory for molecular diagnostics of infectious agents was established in Bo, Sierra Leone with a hybrid solar/diesel/battery system to ensure stable power supply and a satellite modem to enable efficient communication. An array of room temperature stabilization and refrigeration technologies for reliable transport and storage of reagents and biological samples were also tested to ensure sustainable laboratory supplies for diagnostic assays.

Results: The laboratory demonstrated its operational proficiency by conducting an investigation of a suspected avian influenza outbreak at a commercial poultry farm at Bo using broad range resequencing microarrays and real time RT-PCR. The results of the investigation excluded influenza viruses as a possible cause of the outbreak and indicated a link between the outbreak and the presence of *Klebsiella pneumoniae*.

Conclusions: This study demonstrated that by application of a carefully selected set of technologies and sufficient personnel training, it is feasible to deploy and effectively use a broad-range infectious pathogen detection technology in a severely resource-limited setting.

Background

Developing countries in tropical regions of the world are the home for numerous important infectious pathogens [1-3]. Many of these infectious agents may have their reservoirs in domesticated or wild animals [4-8]. Since inhabitants of these countries live in relatively closer contact with animals, than populations of highly developed countries, the chance of transmission of zoonotic infections to humans is much greater [9]. Some of these pathogens not only have severe impact on public health in countries where they are endemic, but may also be rapidly disseminated to non-endemic regions through global transportation networks (air, freight containers), migratory birds, and expanding wildlife trade [10-15]. While outbreaks caused by highly lethal pathogens such as Ebola, Nipah or H5N1 influenza viruses are usually followed by high profile epidemiologic investigations, the everyday infectious disease diagnostics and epidemiological surveillance systems in many of these

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regions are only rudimentary, sub-Saharan Africa being one of the prime examples [16,17]. This situation is compounded by serious shortages of resources and trained personnel capable of performing diagnostic procedures. As a consequence, infectious disease outbreaks in these settings are detected relatively late in their course. A typical example of the problem was the recent yellow fever outbreak that began in October 2010 in Uganda. The outbreak, initially suspected to be caused by Ebola virus, started in early October and was subse- quently misdiagnosed as amoebic dysentery, alcohol poi- soning, and plague before being correctly identified as yellow fever at the end of December. By the time of con- firmation, there were approximately 200 confirmed cases and nearly 50 deaths were recorded [18,19].

Rapid and efficient infectious disease surveillance sys- tems are necessary to improve outbreak management and mitigate the consequences of outbreaks. However, direct replication the infectious outbreak surveillance systems in their current form that exist in developed countries is not practical due to many reasons including absence of detailed maps and lack of basic laboratory in- frastructure needed to support traditional diagnostic sys- tems. Our previous work on participatory mapping and surveying methods has indicated promising solutions to the first problem [20]. The issues related to inefficiency of current infectious disease diagnostics based on culture and simple molecular assays remain a serious challenge. Microbial culture, while still a "gold standard" technique for identification of bacteria, cannot be applied for de- tection of viral pathogens. In addition, microbial culture is labor intensive, time consuming and requires qualified and experienced technicians which are frequently in short supply in these locations. Molecular assays are rapid and sensitive but due to their low level of multi- plexing usually a number of different molecular assays needs to be performed sequentially to achieve definitive diagnosis. This is especially relevant in cases of infec- tious syndromes of diverse etiologies but manifested by similar symptoms [21]. Reliance on these technologies results in significant delays between specimen isolation and pathogen identification. An additional difficulty experienced by many regions of the developing world is the lack of a reliable cold chain capability necessary for transport and preservation of biological samples and diagnostic reagents in hot climate due to unreliable power and a lack of basic refrigeration equipment.

One of the ways to significantly improve the microbial diagnostic capacity in developing countries may be 'leap-frogging' or skipping some stages of technological devel- opment that other countries have passed or are passing through [22,23]. A good example of the "leapfrog" phenomenon is the use of mobile phone technology, which has enabled the communication in villages in developing countries that have never had land phones with their associated expensive infrastructure [24]. In case of microbial diagnostics, developing countries may need to jump directly to broad-range microbial diagnostic systems, which are capable of one-step detection and identification of large number of diverse pathogens in a single, highly automated, assay. Advanced broad-range diagnostic technologies have the potential of making the pathogen identification process simpler and faster leading to more efficient detection and management of infectious disease outbreaks both in humans and animals.

The purpose of this study was to test the feasibility of application of a broad-spectrum diagnostics/surveillance platform for microbial detection in a resource-limited setting. This feasibility study was the result of a collaborative effort between the US Naval Research Laboratory and Njala University, which led to the establishment of a molecular diagnostic laboratory at Mercy Hospital Research Laboratory, Bo, Sierra Leone.

Setting up this type of facility required solving a num- ber of issues typical for developing countries with a trop- ical climate including securing a reliable power supply, implementing cold chain and complementary methods of preserving biological samples and reagents, and enabling efficient communication by setting up an internet linked computer network. This paper describes how a broad spectrum diagnostics system was successfully deployed using a set of "leapfrog" technologies that were found to be critical in establishing an efficient laboratory. The resequencing-microarray-based diagnostic system was subsequently applied for investigation of a suspected avian influenza outbreak at the commercial poultry farm.

Methods

Laboratory setup

The molecular diagnostic laboratory was set up in Mercy Hospital Research Laboratory (MHRL) located in the city of Bo, Sierra Leone. Bo is the second largest city in Sierra Leone and the capital of the Southern Province. The 1,200 square foot laboratory was located on the Mercy Hospital campus in Kulanda Town section of Bo. All equipment, which required temperature within certain limits for proper operation (including PCR instruments, Affymetrix fluidics stations, hybridization ovens and microarray scanner) was located in two air-conditioned rooms. An additional air-conditioned laboratory area detached from the main laboratory building was equipped with a PCR laminar flow hood and used for sample preparation for PCR, RT-PCR and sample processing for resequencing pathogen microarray (RPM) assays.

For cold storage, the laboratory was equipped with two 57 liter AcuTemp AX56L/HemaCool mobile refrigerator/

freezers (AcuTemp, Dayton, OH), four Fridge-Freeze 60 liter portable vaccine refrigeration units (two freezers and two refrigerators) with ability to be powered with 12/24-volt DC or 110/240-volt AC (Fridge-Freeze Inc. San Diego, CA) and one upright Kenmore freezer model 2804 (Sears, Roebuck and Co., Hoffman Estates, IL). HemaCool freezers can be adjusted for freezing (−20°C) or refrigeration (+4°C) and can be run for 16 hrs continuously in the absence of external power on internal batteries.

The power for the laboratory operation was supplied by two hybrid power subsystems, one operating at 230 V, 50 Hz (used for powering European and African made equipment) and another one operating at 120 V, 60 Hz (for powering US made scientific instruments). Both systems relied on combination of solar power with battery storage and diesel generator backup. The 230 V subsystem was additionally connected to municipal power grid and used municipal power when available. The detailed description of the power system and its performance was published previously [25,26].

Communication

A stand-alone solar powered C-band satellite communication system was configured by Satcom Resources (Avon, CO) and deployed at MHRL. Bandwidth (512/128 (kb/s) up/down) was provided by Constellation Networks Corp. (Traverse City, MI) and served up to 20 users simultaneously via intranet across the Mercy Hospital campus. The diagram of the network configuration is included in supplementary data (Additional file 1: Figure S4). Teleconferencing for training purposes and data exchange was conducted using Skype platform (Skype Technologies S.A., Rives de Clausen, Luxembourg).

Ambient temperature stable reagents

Ambient temperature stabilized reagents for PCR, real-time PCR or resequencing microarray protocols were ei- ther obtained commercially or developed in-house using lyophilization techniques (see Additional file 2: Tables S3 and S4 for listing of stabilized reagents tested and used for all molecular diagnostics protocols).

FTA paper - sample stabilization and recovery

Flinders Technology Associates filter paper (FTA paper, Whatman/GE Healthcare, Florham Park, NJ) [27] was used in this work to explore its suitability for dry storage of RNA preparations and for stabilization of field sam- ples collected from poultry. Either Indicating FTA Mini Cards (WB120356, Whatman) or Indicating FTA Classic Cards (WB120206, Whatman) were used depending on the application. An aliquot of RNA samples was spotted on the card or swabs containing field samples were pressed against an FTA card in order to transfer the maximum amount of fluid from swab to the FTA paper. Subsequently the FTA cards were air dried at ambient temperatures for 40 minutes. Dry FTA cards were stored at ambient temperatures unless otherwise indicated.

To recover the nucleic acids for use in diagnostic procedures, the FTA paper embedded samples were processed using the following procedure modified from a protocol developed by Rogers and Burgoyne [28]. Circular punches (1 mm or 3 mm in diameter) of the FTA paper from the areas of sample deposition were taken using Harris Uni-Core punch (Ted Pella Inc., Redding, CA). The punches were placed into 0.5 mL microcentrifuge tubes and incubated for 5 min. with gentle shaking in 200 μl of solution A (4 M LiCl solution in 50% ethanol). The incubation was repeated once with fresh solution A. After incubation, the disks were washed with gentle shaking for 5 min. in 200 μl of solution B (50 mM Tris–HCl solution in isopropanol), then washed twice for 5 min. with 200 μl of 70% ethanol. After the final wash, the samples were air dried at 42°C for at least 30 minutes to remove the traces of ethanol completely.

RNA purification

The RNA was purified from influenza B/Lee/40 preparations (Advanced Biotechnologies, Inc., Columbia, MD) using MasterPure DNA and RNA purification kit (Epicentre Biotechnologies, Madison, WI) using manufacturer recommended protocol.

Testing viral RNA stabilization efficiency on FTA paper

Influenza B RNA samples (5 μl each of 10^6, 10^4 or 10^2 genome copies/μl dilutions) were spotted on Indicating FTA Mini Cards (Whatman). The cards were incubated under one of three conditions: at room temperature 20-25°C (on a laboratory bench), 30°C (incubator) and 30-45°C (outdoors, protected from light) for up to 10 days. Three 1 mm punches were removed from the FTA cards at 24 hours, 3 days, and 10 days). The punches were processed as described above (see "FTA paper - sample stabilization and recovery" section).

Collection of poultry pharyngeal samples

Pharyngeal swabs were collected on March 30th, 2009, using sterile techniques from 136 chickens housed in 9 different poultry farms. The farms were located in Bo and in the vicinity of Freetown (Figure 1), their geographic coordinates were determined using handheld Garmin GPSMAP 60CSx GPS unit (Garmin International Inc., Olathe, KS). Basic farm information, the numbers of samples taken at each farm and general health characteristics of the poultry on farms sampled are included in Table 1. The collected swab samples were stabilized and stored on FTA cards. After transporting to the laboratory in ambient temperatures, the

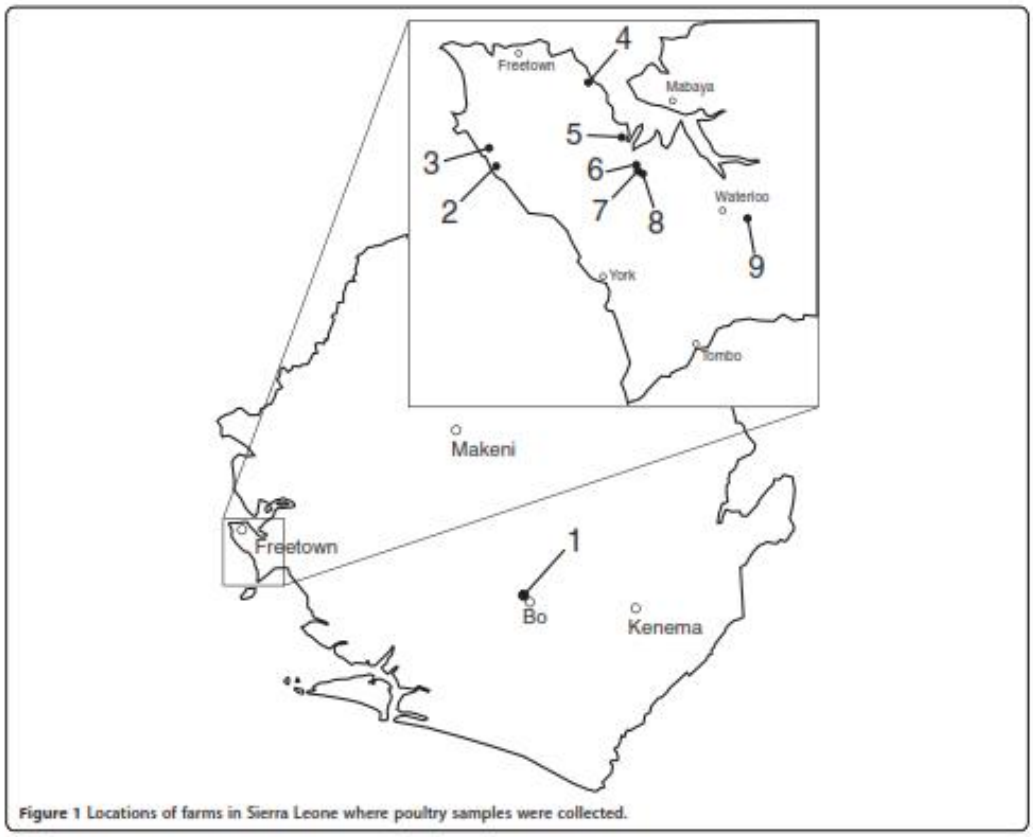

Figure 1 Locations of farms in Sierra Leone where poultry samples were collected.

FTA embedded specimens were placed in −20°C freezer for long term storage.

Resequencing microarrays

The resequencing microarray analysis was conducted using RPM-Flu v. 3.1 (RPM-Flu) and RPM-TEI v. 1.0 microarrays (RPM-TEI) in March 2010. Poultry samples for analysis were selected using a two-stage process. First the samples for which the amount of deposited samples was small (judged by the size of spot with change of color on indicating FTA card) were rejected. The remaining FTA samples were blindly drawn to select 15 samples from Bo farm and 3 samples each from 5 other farms. RPM-Flu v. 3.1 was designed to detect all known subtypes of influenza A viruses and 84 other viral and bacterial respiratory pathogens [29], while the RPM-TEI microarray was designed for detection of a broad range of biothreat agents [30] some of them (such as Lassa virus) endemic to West Africa. Sample processing was conducted as previously described [30-32] with the following modifications related to the use of FTA paper embedded samples. Briefly, reverse transcription using random primers was used to obtain cDNA from RNA templates potentially present in a processed FTA paper disk for each analyzed sample. The resulting mixture was separated from the FTA paper disk and split into four aliquots of equal volume for multiplex PCR reac- tions using either RPM-Flu or RPM-TEI microarray spe- cific primer cocktails. Modified version of this protocol for testing the lyophilized/ambient temperature stabi- lized reagents is included as Supplementary Data.

Pathogen identification was performed using previously developed Computer-Implemented Biological Sequence Identifier (CIBSI) 2.0 software [33]. Although the microar- rays are designed with tiles for specific pathogen targets [29,30,32], they are capable of detection and correct se- quence determination of targets differing by up to 15% from the sequence present on the microarray. This allows for detection of target variants and near-neighbor discrimination.

Table 1 Farm and chicken sample information

No.	Farm location		No. of sheds	No. of samples collected	Sample designations	Condition of chickens
	Town	Coordinates				
1	Bo	7°57.911'N 11°44.767'W	1	40	B1-B40	Outbreak affecting 15% of chickens
2	Hamilton	8°23.275'N 13°15.453'W	4	20	H1-H20	All healthy
3	Oogu	8°24.256'N 13°15.612'W	1	16	O1-O16	All healthy
4	Wellington	8°27.222'N 13°10.242'W	3	12	W1-12	Some chickens sick
5	Allen Town	8°24.266'N 13°8.742'W	11	16	A1-16	All healthy
6	Hastings	8°22.776'N 13°8.094'W	1	8	HA1-HA8	All healthy
7	Hastings	8°22.622'N 13°7.997'W	1	4	HB1-HB4	All healthy
8	Hastings	8°22.403'N 13°7.925'W	2	8	HC1-HC8	All healthy
9	Joe Town	8°19.549'N 13°2.535'W	3	12	J1-12	Some chickens sick

Reverse transcription and real time PCR (RT-PCR)

In order to detect influenza B RNA in testing RNA stabilization on FTA 1 mm processed disks were placed in a 0.5 mL PCR tube, and subjected to one step reverse transcription and PCR (RT-PCR) using Qiagen OneStep RT-PCR Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Positive controls were prepared using liquid preparations of influenza B RNA stored at −20°C. The RT-PCR reaction was run in 25 μl total volume using previously published BMA-F1 and BMA-R1 primers for detection of influenza B [31] and the following thermal cycling protocol: 50°C for 30 min; 95°C for 15 min.; 40 cycles of 94°C for 30 sec., 54°C 30 sec., 72°C 30 sec.; 72°C 10 min. The expected amplicon was a 162 bp segment of the influenza B matrix gene. The amplification results were analyzed using 2% TAE agarose gels containing ethidium bromide. The bands were visualized in UV light and images captured using UVP BioDoc-It System, model M-20 (UVP, Upland, CA).

Real-time RT-PCR for universal detection of influenza A (based on detection of fragment of the Matrix gene) was performed using previously published PCR primers: MatrixF1 and MatrixR1 [34]. A preparation of influenza A H3N2 control strain was used as a positive control, water and blank processed FTA paper discs were used as negative controls. Reverse transcription (RT) was performed using AccuPower Cyclescript RT premix (Bioneer, Alameda, CA). The total volume of the RT reaction was 20 μl containing 500 nM each of forward and reverse primers. The reaction mixture was subjected to 12 cycles of incubation at 25°C for 1 min. and 50°C for 4 min. and single final incubation at 95°C for 5 min. The real-time PCR amplification reactions were conducted using SsoFast EvaGreen reaction mix (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. The reaction was carried out in 20 μl total volume containing 500 nM of each primer with 2 μl of the RT reaction mixture as a template. The thermal cycling and fluorescent signal detection was performed in a CFX96 real-time PCR detection system (Bio-Rad Laboratories) with the following thermal cycling conditions: initial incubation at 98°C for 2 min. followed by 40 cycles of 98°C for 2 sec. and 60°C for 5 sec. The amplification cycle was followed by melting curve analysis. The results were analyzed with CFX Manager software ver. 1.5.534.0511 (Bio-Rad Laboratories).

The protocols for RT-PCR and real-time RT-PCR for detection of influenza A using ambient temperature stabilized reagents are described in Supplementary Data (Additional file 2), and the reagents used with these protocols are listed in (Additional file 2): Tables S3 and S4.

Results and discussion

Laboratory operation

This study was intended to identify the suite of technologies necessary to deploy and successfully apply an advanced, "leapfrogging", technology for broad range pathogen identification in a severely resource-limited

Table 2 Technologies applied to deploy broad-range infectious pathogen diagnostics

Problem	Solution/s applied
Unreliable power	Efficient hybrid solar/diesel power system
Lack of "cold chain"	On-site refrigeration
	FTA paper for sample collection, transport and storage
	Ambient temperature stabilized reagent sets
	Delivery of regular reagents on dry-ice
Lack of efficient communication	Wired/wireless, hi-speed campus network connected with Internet via satellite
Inadequate personnel expertise	Hands-on training in reference laboratory (NRL) and on-site.
	Remote technical support by email and Skype videoconferencing.

setting. The typical major challenges in setting up a laboratory in a developing country are listed in Table 2. They needed to be addressed in order to make the laboratory operational and capable of reliably running high qual- ity molecular diagnostic protocols. While the general class of solutions was easy to recognize, the actual solution implemented depended intimately on the local conditions.

Stable power

One of the most significant difficulties in establishing stable operation of the molecular diagnostics laboratory in Bo was inadequate power necessary to run laboratory equipment. Due to the reliance on hydroelectric power generation in Sierra Leone, the availability of municipal electricity (supplied by Bo/Kenema Power Service – BKPS) varies throughout the year and power is mostly unavailable during the dry season spanning from November to April. Even when available, the electricity is of poor quality due to inadequate design of electrical grid and is not suitable for powering sensitive scientific equipment [26]. To overcome this problem an innovative hybrid power system composed two self-contained local grids with two hybrid power subsystems (230 V, 50 Hz and
120 V, 60 Hz) was designed and deployed [25,26]. The system combined solar power generation with battery storage and diesel generator backup to provide reliable power for both basic laboratory infrastructure (e.g. lights, air conditioners) as well as sensitive scientific instruments (e.g. PCR cyclers, GenChip scanner).

Personnel training

Training is another key component of a successful laboratory set up. The laboratory personnel have undergone 10-week training in basic molecular biology diagnostic techniques including various PCR, and resequencing microarray technologies at Naval Research Laboratory (NRL) located in Washington, DC. A follow up training was conducted in Bo by NRL personnel. Personnel from MHRL also obtained scanner maintenance and calibration training from Affymetrix to ensure proper functioning of the scanner since this was the most delicate instrument of the system and needed to be periodically recalibrated. The proficiency of trained personnel was successfully demonstrated by conducting the investigation of an outbreak at the poultry farm that is described below. In addition to initial training, the NRL personnel were remotely supporting MHRL scien- tists taking advantage of the Internet connected com- puter network and Skype based videoconferencing.

Stabilization of field samples and molecular biology reagents

While the stable power and use of freezers and refrigerators with battery backup solved the problem of storing perishable reagents and samples in the laboratory, it did not address the issues related to preservation samples collected in the field. Transport of temperature sensitive reagents needed for diagnostic procedures was also a significant problem due to unavailability of commercial "dry-ice" refrigerated transport service in Sierra Leone. To overcome these difficulties we explored applicability of two technologies: FTA paper for field sample collection and transport as well as stabilization of molecular biology reagents by freeze drying or related techniques.

FTA paper

FTA paper is a well-established technology for ambient temperature preservation of nucleic acids and was designed to protect nucleic acids of the stored sample from degradation caused by nucleases, oxidation, UV light and other processes [35,36]. FTA paper also rapidly inactivates pathogens making the infectious samples safer to handle by untrained personnel [35-37]. While it was shown that FTA cards are able to adequately store DNA samples at room temperature for at least 17 years without significant degradation [38,39], only limited data is available on the stability of RNA on FTA paper, especially when stored at elevated temperatures [28,35,40]. A series of experiments was conducted to find out if FTA technology might be suitable for short-term preservation of samples containing RNA viruses at high ambient temperatures characteristic for a tropical country such as Sierra Leone. Influenza B was used as a model organism for testing. Three different concentrations of influenza B RNA were spotted on FTA Minicards and stored at various temperature conditions for 1–10 days. The higher concentration of RNA (10^6 and 10^4 copies/μl) was consistently detectable after 10 days even when incubated at the highest tested temperatures (Table 3 and Additional file 1: Figures S1-S3). Although the lower concentration of RNA (10^2 copies/μl) was not detectable in more than half of the analyzed samples, the lack of detection did not seem to correlate with storage conditions. These results indicated that in addition to the established capability to maintain stable DNA, the FTA cards could also be used to collect and store RNA samples for a time frame sufficient to transport and test samples. Inconsistent results of recovery of low concentrations of influenza B from FTA paper most likely reflected the phenomenon of dilution of samples deposited on FTA or inefficient recovery rather than degradation of the RNA.

Reagent stabilization

In an effort to overcome the problems with the delivery of temperature sensitive reagents to Sierra Leone, we made an attempt to design molecular diagnostic protocols taking advantage of ambient temperature stabilized reagents. Commercially available stabilized reagents were

Table 3 Efficiency of detection of Influenza B RNA stored on FTA paper incubated at elevated temperatures[1]

Influenza B RNA concentration (copies/microliter)	Duration of incubation (hours) temperatures									Incubation (°C)
	24			72			240			
10^6	+	+	+	+	+	+	+	+	+	20-25 (RT)
	+	+	+	+	+	+	+	+	+	30
	+	+	+	+	+	+	+	+	+	30-45 (outdoors)
10^4	+	+	+	+	+	+	+	+	+	20-25 (RT)
	+	+	+	F	+	+	+	+	+	30
	+	+	+	+	+	+	+	+	+	30-45 (outdoors)
10^2	–	–	–	+	+	F	–	F	F	20-25 (RT)
	–	–	–	F	F	–	–	–	–	30
	F	F	F	–	F	F	–	–	–	30-45 (outdoors)
10^6 (control RNA)	+			+			+			–20 (freezer)
10^2 (control RNA)	+			+			+			–20 (freezer)

[1]Each experiment for particular combination of sample concentration and temperature was run in triplicate. Plus sign indicates that RT-PCR detection produced strong band of expected size that was observed on a gel; F indicates a faint band and a minus sign indicates absence of an amplification product detectable by visual inspection on a gel.

used for reverse transcription, PCR, and real-time PCR together with modified protocols optimized for use with these regents. However in case of RPM platform, the stabilized reagents were developed in-house since there were no commercially stabilized reagents available. These reagents were developed by adaptation of previously published methods [41-43]. Details of the protocols, commercial reagents tested and custom reagent composition and stabilization procedures are described in supplementary data (Additional file 2).

While these ambient-temperature-stabilized reagent sets were very stable in high ambient temperatures, the testing results showed that diagnostic assays using these reagents were significantly less sensitive than traditional reagents (data not shown). Due to financial and time constraints, optimization of the stabilized reagents was not pursued. While the stabilized reagents were not used for the subsequent epidemiological investigation, the re- agent stabilization technology has a great potential to make the molecular diagnostics more accessible in developing countries by eliminating the cold chain, greatly lowering power requirements that are dominated by refrigeration equipment and making the diagnostic protocols significantly less complex and error prone [41-43] and should be further explored in future.

As an alternative, reagents were transported as carry- on "dry ice" package in accordance with all airline regu- lations. The packages were prepared with sufficient amount of dry ice for 48 hours and passed through x-ray examination. Testing conducted with these reagents showed no noticeable difference in performance. Since US based personnel overseeing Mercy Hospital travel regularly (every 1–2 months) to the site, it was possible to ship reagents to maintain operation of the molecular diagnostics laboratory for sustained periods of time.

Poultry outbreak investigation

To test the operational capabilities of MHRL molecular diagnostics laboratory broad-range microbial detection assays were used to investigate an outbreak that occurred at one of the few commercial poultry farms located in Bo. The concern was that the outbreak might have been caused by a highly pathogenic influenza virus which might be potentially transmitted to farm workers or poultry kept by individual owners. This is a significant risk especially in developing countries where it is a common practice to keep chickens in close proximity to the household and potentially expose the whole families to the poultry pathogens.

Two different assays, RPM-Flu and RPM-TEI, were used to analyze the outbreak samples. The analysis strategy relied on using microarray based broad-range detection assay to analyze just a small percentage of collected samples and follow up using single specific PCR based assays for larger numbers of samples based on the results of microarray analysis. Therefore only 15 randomly selected samples (see methods for selection process details) out of a total of 40 pharyngeal swab samples collected in the farm located in Bo and preserved on FTA paper were tested using resequencing assays. For comparison, 15 additional samples were selected from 5 other farms located in the Freetown area which housed mostly healthy chickens were also analyzed using the same microarrays. The results of RPM

Table 4 Results of pathogen detection using RPM-Flu 3.1 microarray

Farm	Sample	Most likely ID*
1	B1	K. pneumoniae, P. stutzeri
B2	K. pneumoniae, P. stutzeri B3	K. pneumoniae
B4	no detection B5	no detection B11 no detection
B12	K. pneumoniae, P. aeruginosa, Staph. (mecA gene) B13	K. pneumoniae, Pseudomonas
B14	K. pneumoniae, P. aeruginosa, Staph. (mecA gene) B15	K. pneumoniae, P. putida, Staph. (mecA gene)
B25	K. pneumoniae, Pseudomonas	
B26	K. pneumoniae	
B27	K. pneumoniae, P. putida	
B28	K. pneumoniae, P. aeruginosa, Staph. (mecA gene) B35	no detection
2	H2	no detection
H3	no detection	
H4	(P. aeruginosa or M. catarrhalis)	
6	HA5	(P. aeruginosa or M. catarrhalis)
HA6	no detection	
HA7	Pseudomonas	
5	A1	no detection
A2	P. putida	
A16	no detection	
9	J2	P. aeruginosa, E. sakazakii
J7	no detection	
J10	no detection	
4	W3	K. pneumoniae
W4	P. aeruginosa	
W6	(Pseudomonas or Moraxella or Methylobacillus)	

*Most likely ID was determined using CIBSI algorithm and based on similarity analysis of the sequences obtained from the microarray with sequences deposited GenBank at the time of conducting the analysis (April 2010). Abbreviations used: K. pneumoniae = Klebsiella pneumoniae, P. stutzeri = Pseudomonas stutzeri, Staph. = Staphylococcus spp., P. aeruginosa = Pseudomonas aeruginosa, P. putida = Pseudomonas putida, M. catarrhalis = Moraxella catarhalis, E. sakazakii = Enterobacter sakazakii. Organism names enclosed in parentheses denote result of hybridization of a single microarray tile, which cannot be unambiguously identified based on the obtained sequence.

microarray analysis of selected samples are summarized in Table 4 and described below.

Bacterial pathogens

While no viral pathogens were detected using RPM assays, a number of bacterial pathogens including Klebsiella pneumoniae and several Pseudomonas species were found in analyzed samples.

An assortment of closely related Pseudomonas species (P. stutzeri, P. aeruginosa, P. putida) or undefined Pseudomonas was detected in 16 of the 30 analyzed samples. The ubiquitous presence of Pseudomonas spp., a known opportunist organisms colonizing the avian respiratory tract [44], indicated that it played no significant role in the outbreak. K. pneumoniae on the other hand was found mostly in the samples from farm in Bo (73%, 12 out of the 15 analyzed samples), while only one sample from farms outside of Bo was positive for this organism (7%, Table 4). This result suggested a link between the outbreak and the presence of K. pneumoniae. Although K. pneumoniae is usually considered an environmental contaminant, it may sporadically cause embryo mortality, yolk sac infections and mortality in young chickens, turkeys and ostriches. In addition, the concurrent infection of young turkeys with K. pneumoniae is known to increase the severity of respiratory disease caused by other pathogens [44]. The presence of K. pneumoniae in the majority of outbreak samples suggested that it is an opportunist pathogen colonizing/coinfecting sick chickens and increasing the severity of infection caused by another (unidentified) pathogen. This notion was also supported by the fact that higher percentages of outbreak samples were testing positive for more than one pathogen simultaneously (60% vs. 6% of healthy samples).

In addition to previously mentioned bacteria, the RPM-Flu microarray also detected mecA (methicillin resistance) gene in four samples collected from the farm in Bo. The mecA gene is responsible for staphylococcal resistance to a broad range of β-lactam antibiotics [45]. While mecA carrying bacteria in chickens were reported before [46], it is unknown if the staphylococci carrying this gene were acquired by poultry from humans or the spread of this resistance mechanism was purely zoonotic. Nonetheless, the prevalence of mecA gene (at least 10% of analyzed samples collected in Bo) in the staphylococci colonizing/infecting the analyzed population of chickens is a cause of concern due to a potential of spread to humans and warrants further study.

Influenza virus

Lack of positive detection of influenza in all samples tested with RPM-Flu assay indicated that it is unlikely that the outbreak was caused by any known strain of influenza virus as it was initially suspected. To independently confirm the RPM-Flu assay and expand the results of influenza A detection, all 136 collected samples were analyzed using a published real-time RT-PCR assay [34]. This assay, amplifying a conserved segment of influenza matrix gene to detect influenza A regardless of serotype,

also did not detect the presence of this virus in any of the analyzed samples (data not shown).

Based on the obtained results, it was impossible to exclude the possibility that the outbreak was caused by one of the major poultry respiratory pathogens (such as avian paramyxovirus type 2 [47] or avian metapneumovirus [48]) that are not represented on the RPM-Flu microarray, since this assay was targeted for detection of human pathogens.

Although, the conclusive determination of the cause of the outbreak and the roles of particular identified patho- gens was not possible based on the available data, the out- break investigation demonstrated potential usefulness of the broad-range microbial detection technology in future investigations. The recent emergence and spread of highly pathogenic avian influenza strains has raised concern about outbreaks in poultry farms. As a result, the local health authorities usually treat poultry farm outbreaks as a potential deadly threat to humans, and order all the birds in the affected farms to be culled as a precaution. This practice results in a very significant economic burden to farm owners in developing countries and it may be avoid- able. In this study, the delay of completion of the molecu- lar analysis (approx. 11 months) caused by problems associated with logistics of reagent delivery did not enable us to prevent the slaughtering of the flock, however, the study indicated that application of broad–spectrum mi- crobial diagnostics might make it possible in future out- breaks. While cost of conducting a single RPM assay (approx. $100-200) is too high for routine diagnostics in resource-limited settings, the technology may be a cost ef- fective way for national surveillance of avian influenza and other important human pathogens. The power of a single set of RPM assays allows detection of a panel of pathogens that would otherwise require a full national reference la- boratory infrastructure, which would costs millions of USD. In the case of this project the total cost of setting up the laboratory to run RPM based assays was approxi- mately $250 thousand, including the building for labora- tory set up, power equipment, scientific instruments, telecommunication infrastructure and training.

Conclusions

The project has shown that successful deployment and application of an advanced diagnostic technology in the conditions of low-resource tropical country is feasible. One of the most important outcomes of this effort was identification of a set of technologies that are needed to achieve this goal in an extremely challenging environ- ment. Laying this groundwork will help us and others to build infectious pathogen diagnostic capacity in develop- ing world in an efficient way by taking advantage of technological "leapfrogging".

Additional files

Additional file 1: Supplementary Data Figures.

Additional file 2: Supplementary Data [30,31,34,49].

Competing interests

APM, BL and DAS are inventors of four US patents, and one pending patent application that are related to RPM technologies. These authors also receive royalty payment from Tessarae LLC (Potomac Fall, VA, USA), which licenses the RPM technologies for commercial purposes.

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Authors' contributions

RA and TAL were involved in study conception, data collection, and drafting of the manuscript. APM was involved in the study conception, data analysis, and critical manuscript revision. ASB and AJS were involved in GIS data collection and manuscript revision. DHJ and UB were involved in poultry sample collection, molecular analyses and manuscript revision. BRB was involved in FTA card testing, and drafting part of the manuscript. MA was involved in poultry sample collection, and manuscript revision. BMK was involved in study conception and manuscript revision. NCL, BL and TAL were involved in MHRL personnel training. BL was involved study conception, data analysis and critical manuscript revision. DAS was involved in study conception and manuscript revision. All authors have read and approved of the final manuscript.

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References

1. Kebede S, Duales S, Yokouide A, Alemu W: Trends of major disease outbreaks in the African region, 2003–2007. East Afr J Public Health 2010, 7(1):20–29.
2. Bausch DG, Demby AH, Coulibaly M, Kanu J, Goba A, Bah A, Conde N, Wurtzel HL, Cavallaro KF, Lloyd E, et al: Lassa fever in Guinea:I. Epidemiology of human disease and clinical observations. Vector Borne Zoonotic Dis 2001, 1(4):269–281.
3. Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C: Estimates of world-wide distribution of child deaths from acute respiratory infections. Lancet Infect Dis 2002, 2(1):25–32.
4. Merianos A: Surveillance and Response to Disease Emergence. In Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission. Volume 315, edn. Edited by Childs JE, Mackenzie JS, Richt JA. Berlin Heidelberg: Springer; 2007:477–509.
5. Ozer N: Emerging vector-borne diseases in a changing environment. Turk J Biol 2005, 29:125–135.

6. Meng XJ: Hepatitis E virus: animal reservoirs and zoonotic risk. Vet Microbiol 2010, 140(3–4):256–265.
7. Fichet-Calvet E, Lecompte E, Koivogui L, Soropogui B, Dore A, Kourouma F, Sylla O, Daffis S, Koulemou K, Ter Meulen J: Fluctuation of abundance and Lassa virus prevalence in Mastomys natalensis in Guinea, West Africa. Vector Borne Zoonotic Dis 2007, 7(2):119–128.
8. Chomel BB, Sun B: Zoonoses in the bedroom. Emerg Infect Dis 2011, 17(2):167–172.
9. Maudlin I, Eisler MC, Welburn SC: Neglected and endemic zoonoses. Philos Trans R Soc Lond B Biol Sci 2009, 364(1530):2777–2787.
10. Tatem AJ, Rogers DJ, Hay SI: Global Transport Networks and Infectious Disease Spread. In Global Mapping of Infectious Diseases Methods, Examples and Emerging Applications. Edited by Simon I, Hay AG, David JR. New York: Academic; 2006:293–343. Rollinson D, Hay SI(Series Editors):: Advances in Parasitology, vol 62.
11. Reiter P: The standardised freight container: vector of vectors and vector-borne diseases. Rev Sci Tech 2010, 29(1):57–64.
12. Webster CH: Airline operating realities and the global spread of infectious diseases. Asia-Pacific journal of public health/Asia-Pacific Academic Consortium for Public Health 2010, 22(3 Suppl):137S–143S.
13. Lines J: Chikungunya in Italy. BMJ 2007, 335(7620):576.
14. Tatem AJ, Rogers DJ, Hay SI: Estimating the malaria risk of African mosquito movement by air travel. Malar J 2006, 5:57.
15. Karesh WB, Cook RA, Bennett EL, Newcomb J: Wildlife trade and global disease emergence. Emerg Infect Dis 2005, 11(7):1000–1002.
16. Bates I, Maitland K: Are laboratory services coming of age in sub-Saharan Africa? Clin Infect Dis 2006, 42(3):383–384.
17. Nkengasong JN, Nsubuga P, Nwanyanwu O, Gershy-Damet GM, Roscigno G, Bulterys M, Schoub B, DeCock KM, Birx D: Laboratory systems and services are critical in global health: time to end the neglect? Am J Clin Pathol 2010, 134(3):368–373.
18. FluTrackers.com: Uganda: Hemorrhagic fever outbreak- Yellow Fever.,. http://www.flutrackers.com/forum/showthread.php?t=155125.
19. World Health Organization: Yellow fever in Uganda.; http://www.who.int/csr/don/2011_01_19/en/index.html.
20. Ansumana R, Malanoski AP, Bockarie AS, Sundufu AJ, Jimmy DH, Bangura U, Jacobsen KH, Lin B, Stenger DA: Enabling methods for community health mapping in developing countries. Int J Health Geogr 2010, 9:56.
21. Anthony RM, Brown TJ, French GL: Rapid diagnosis of bacteremia by universal amplification of 23 S ribosomal DNA followed by hybridization to an oligonucleotide array. J Clin Microbiol 2000, 38(2):781–788.
22. Goldemberg J: Leapfrog energy technologies. Energy Policy 1998, 26(10):729–741.
23. Watson J, Sauter R: Sustainable innovation through leapfrogging: a review of the evidence. International Journal of Technology and Globalisation 2011, 5(3–4):170–189.
24. Kwankam SY, Ningo NN: Information Technology in Africa: A Proactive Approach and the Prospects of Leapfrogging Decades in the Development Process. In Inet97. Kuala Lumpur, Malaysia: Internet Society; 1997.
25. Jacobus HS, Lin B, Jimmy DH, Ansumana R, Malanoski AP, Stenger DA: Evaluating the impact of adding energy storage on the performance of a hybrid power system. Energy Conversion and Management 2011, 52(7):2604–2610.
26. Jacobus HS: Solar-Diesel Hybrid Power System Optimization and Experimental Validation. In MS thesis.: University of Maryland; 2010.
27. Burgoyne LA: Solid medium and method for DNA storage. In Edited by USPTO, vol. 5496562. USA: Flinders Technologies Pty Ltd; 1996.
28. Rogers CD, Burgoyne LA: Reverse transcription of an RNA genome from databasing paper (FTA(R)). Biotechnology and Applied Biochemistry 2000, 31(Pt 3):219–224.
29. Lin B, Malanoski AP, Wang Z, Blaney KM, Long NC, Meador CE, Metzgar D, MC A, YS L, MM R, et al: Universal detection and Identification of avian Influenza using resequencing microarrays. J Clin Microbiol 2009, 47(4):988–993.
30. Leski TA, Lin B, Malanoski AP, Wang Z, Long NC, Meador CE, Barrows B, Ibrahim S, Hardick JP, Aitichou M, et al: Testing and validation of high density resequencing microarray for broad range biothreat agents detection. PLoS ONE 2009, 4(8):e6569.
31. Lin B, Blaney KM, Malanoski AP, Ligler AG, Schnur JM, Metzgar D, Russell KL, Stenger DA: Using a resequencing microarray as a multiple respiratory pathogen detection assay. J Clin Microbiol 2007, 45(2):443–452.
32. Wang Z, Malanoski AP, Lin B, Kidd C, Long NC, Blaney KM, Thach DC, Tibbetts C, Stenger DA: Resequencing microarray probe design for typing genetically diverse viruses: human rhinoviruses and enteroviruses. BMC genomics 2008, 9(1):577.
33. Malanoski AP, Lin B, Wang Z, Schnur JM, Stenger DA: Automated identification of multiple micro-organisms from resequencing DNA microarrays. Nucleic Acids Res 2006, 34(18):5300–5311.
34. Carr MJ, Gunson R, Maclean A, Coughlan S, Fitzgerald M, Scully M, O'Herlihy B, Ryan J, O'Flanagan D, Connell J, et al: Development of a real-time RT- PCR for the detection of swine-lineage influenza A (H1N1) virus infections. J Clin Virol 2009, 45(3):196–199.
35. Whatman: Whatman FTA for Total RNA. Whatman 2004, 51643:1–2.
36. Whatman: Whatman FTA DNA protection. Whatman 2006, 51677:1–2.
37. Abdelwhab EM, Luschow D, Harder TC, Hafez HM: The use of FTA((R)) filter papers for diagnosis of avian influenza virus. J Virol Methods 2011, 174(1–2):120–122.
38. Smith LM, Burgoyne LA: Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper. BMC ecology 2004, 4:4.
39. Whatman: FTA Nucleic Acid Collection, Storage and Purification.; http://www.whatman.com/FTANucleicAcidCollectionStorageandPurification.aspx.
40. Natarajan P, Trinh T, Mertz L, Goldsborough M, Fox DK: Paper-based archiving of mammalian and plant samples for RNA analysis. Biotechniques 2000, 29(6):1328–1333.
41. Chua AL, Elina HT, Lim BH, Yean CY, Ravichandran M, Lalitha P: Development of a dry reagent-based triplex PCR for the detection of toxigenic and non-toxigenic Vibrio cholerae. J Med Microbiol 2011, 60(Pt 4):481–485.
42. Klatser PR, Kuijper S, van Ingen CW, Kolk AH: Stabilized, freeze-dried PCR mix for detection of mycobacteria. J Clin Microbiol 1998, 36(6):1798–1800.
43. Aziah I, Ravichandran M, Ismail A: Amplification of ST50 gene using dry-reagent-based polymerase chain reaction for the detection of Salmonella typhi. Diagn Microbiol Infect Dis 2007, 59(4):373–377.
44. Barnes HJ: Miscellaneous and sporadic bacterial infections. In Diseases of poultry. 11th edition. Edited by Saif YM. Ames: Blackwell Publishing Company; 2003:845–862.
45. Berger-Bachi B: Genetic basis of methicillin resistance in Staphylococcus aureus. Cellular and Molecular Life Sciences 1999, 56(9–10):764–770.
46. Kawano J, Shimizu A, Saitoh Y, Yagi M, Saito T, Okamoto R: Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. J Clin Microbiol 1996, 34(9):2072–2077.
47. Alexander DJ: Avian Paramyxoviruses 2–9. In Diseases of poultry. 11th edition. Edited by Saif YM. Ames: Blackwell Publishing Company; 2003:88–92.
48. Gough RE: Avian Pneumoviruses. In Diseases of poultry. 11th edition. Edited by Saif YM. Ames: Blackwell Publishing Company; 2003:92–100.
49. Colaco C, et al: Extraordinary stability of enzymes dried in trehalose: simplified molecular biology. Biotechnology (N Y) 1992, 10(9):1007–1011.

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Enabling methods for community health mapping in developing countries

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Abstract

Background: Spatial epidemiology is useful but difficult to apply in developing countries due to the low availability of digitized maps and address systems, accurate population distributions, and computational tools. A community-based mapping approach was used to demonstrate that participatory geographic information system (PGIS) techniques can provide information helpful for health and community development.

Results: The PGIS process allowed for the rapid determination of sectional (neighborhood) boundaries within the city of Bo, Sierra Leone. When combined with data about hospital laboratory visits, a catchment area for one hospital in Bo could be established. A survey of households from within the catchment area determined that the average population per household (about 6 individuals) was similar to that found in the 2004 census. However, we also found that the average house was inhabited by more than one household, for an average of 17.5 inhabitants per residential building, which is critical information to know when estimating population size using remote imagery that can detect and enumerate buildings.

Conclusions: The methods developed in this paper serve as a model for the involvement of communities in the generation of municipal maps and their application to community and health concerns.

Background

Low-income countries would benefit significantly from the expanded use of Geographic Information Systems (GIS) in the analysis of disease distribution, since GIS could provide more accurate information about disease incidence and prevalence rates and would allow for better allocation of the limited resources available for public health [1-5]. However, these areas face significant barriers to the implementation of GIS for spatial epidemiology, due both to a lack of disease data (because of limitations in disease detection and reporting systems) and to the non-existence of detailed maps, especially in areas affected by conflict, population displacement, and rapid urbanization. Without accurate population, disease, and spatial data, it will not be possible to implement effective surveillance systems in these countries. Increasing the accuracy of these measures will require an improvement in data collection and management systems as well as a significant increase in the ability of scientists in low-income countries to apply epidemiological, laboratory, and GIS techniques to local health concerns [6-8].

Freely-available mapping and analysis tools and free or low-cost data and image sources (such as Google Earth) can be a greatly beneficial starting point for generating basic map features in areas where this information is not already available. However, effective disease surveillance requires that physical geographic information be supplemented by data about social and population factors that can be mapped at a fine scale. Combining information about distances from water, waste disposal areas, swampy areas, and other physical characteristics as well as information on population density, economic factors, and the location of health resources provides a more complete picture of health and disease by providing information about potential mitigating and debilitating factors. Even simple information about the location of patients' homes and the characteristics of those dwellings can provide helpful information about both physical

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and socioeconomic parameters that can be incorporated into a health GIS, information that is rarely available in low-income countries with limited access to technology. As the amount of remote imagery available for use in lower-income areas is expanding, so is the need for ground-truth validation of these high-resolution maps, which must be completed before advanced GIS analysis can be conducted [9-14].

Methods that engage and empower communities and allow local residents to incorporate their knowledge into the production of a GIS are a promising emerging approach for assembling ground truth data. Public Participation Geographic Information Systems (PPGIS) emerged in the 1980s as a formal method to involve the "public" in the creation and use of geographic information [15-17]. PPGIS and related approaches, such as participatory GIS (PGIS), community integrated GIS, and GIS for participation, have been adapted for use in a variety of settings with various levels of local participation, different types of communities, and a range of intended applications [17-20]. In each of these models, the goal is to integrate local knowledge with "expert" data and techniques.

In this study, we developed and used participatory methods to provide a low-cost solution to address mapping issues in Bo, Sierra Leone, and to begin populating a GIS with population statistics that can be used to address many community issues. Our specific aims were as follows: (1) to create a map of the sections (neighborhoods) of Bo by employing a participatory mapping method, which included interviews of knowledgeable long-term local residents and consultation with municipal authorities in the Bo City Council and local officials and (2) to estimate total population in a few sections by combining data from the map, the 2004 census, and population data obtained from household surveys. As part of the first aim and an example of how maps can be applied to health-related research, an analysis of visits to the Mercy Hospital Laboratory was performed using hospital records. Future studies will use the information from the map and surveys of households sampled from residential buildings identified on the map to assist with the initiation of an active infectious disease surveillance system and the analysis of the social and environmental factors contributing to the incidence and prevalence of diseases.

Results

Sectional mapping

Our first goal was to use a participatory process to create a map of the boundaries for each section within the city of Bo, which is the second largest city in Sierra Leone and the urban center of Bo District (the equivalent of a state or province). Although sections are formally-recognized and distinct areas of the city, no official map of the sections in Bo has been made since 1964, when the Directorate of Overseas Surveys (D.O.S.) produced a map of Bo with sectional information and street addresses for Sierra Leone's Ministry of Lands, Housing, Country Planning, Forestry and the Environment (MLCPE). This map is now obsolete due to the significant growth of the city over the past forty years and to a civil war from 1991 to 2002 that destroyed much of the country's infrastructure and capacities. Now that peace has been re-established, new maps are required to provide accurate current information. The Development Assistance Coordination Office (DACO) and the Sierra Leone Information System (SLIS) have developed a map of main roads and landmark buildings from 2002 Ikonos imagery, but this map does not show the location of the sections of Bo city.

The participatory process employed in this study resulted in the mapping of 68 sections within the 30.1 km^2 area encompassed by the city of Bo. Each section has a unique shape (Figure 1) and size (Table 1), with sectional areas ranging from 0.02 km^2 (Toubu) to 2.33 km^2 (Bo Government Reservation). The section map provides organizational information about the city of Bo, and additional information, such as the locations of hospitals and large clinics (Figure 1), can be displayed at the sectional level.

Catchment area study

The sectional map will be useful for a variety of applications, including public planning and social and health research. A brief illustration of this applicability involves the use of the map to define a catchment area for one of the hospitals in Bo, Mercy Hospital, which is on the north side of the city (Figure 1). A summary of the residents of Bo who visited Mercy Hospital Laboratory for clinical testing and provided the name of their home section is shown in Table 1. (Patient information was extracted from the hospital's computerized database.) Figure 1 shows the home sections for those 797 patients (which represent only 52% of the 1810 total laboratory patients) using a darker grey to represent sections that are home to more Mercy Hospital patients. As might be expected, the majority of laboratory patients (n = 274) live in the section in which Mercy Hospital is located, Kulanda Town. The number of patients is smaller in other sections (n = 0 to 55), and the number decreases as the distance from the hospital becomes greater. The section map will make it easier for future patients to identify their home neighborhoods, which will increase the proportion of patients who can be included in the GIS.

Figure 1 also shows that 13 sections have half or more of their area located within 1 km of Mercy Hospital.

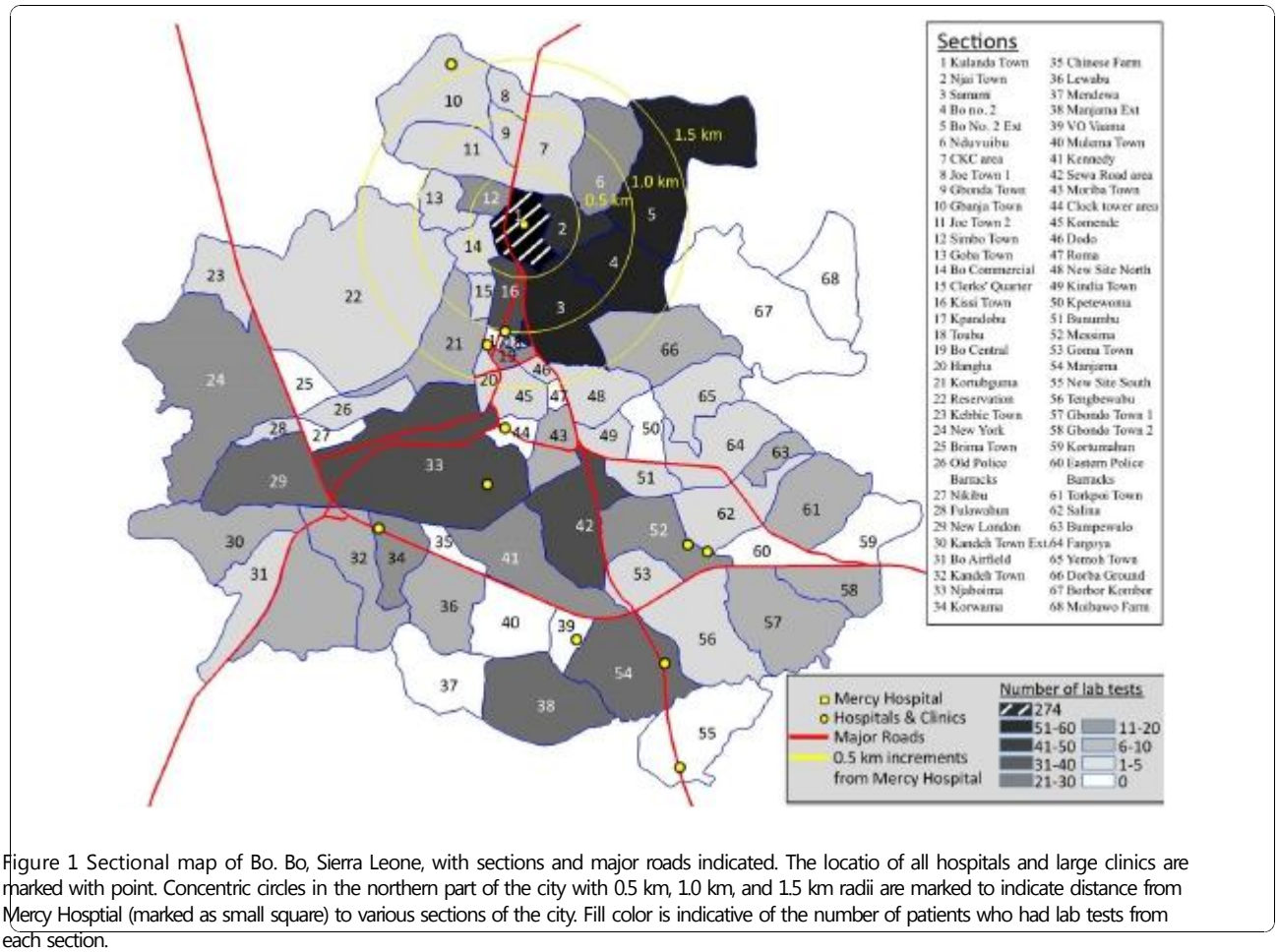

Figure 1 Sectional map of Bo. Bo, Sierra Leone, with sections and major roads indicated. The locatio of all hospitals and large clinics are marked with point. Concentric circles in the northern part of the city with 0.5 km, 1.0 km, and 1.5 km radii are marked to indicate distance from Mercy Hosptial (marked as small square) to various sections of the city. Fill color is indicative of the number of patients who had lab tests from each section.

From Table 1, it can be determined then that 509 (64%) of hospital patients with a known residential section live in one of these nearby sections, which represent only 12.5% (3.76 km^2) of the total area of Bo. This demonstrates that the pool of people who normally visit Mercy hospital was strongly associated with distance from the hospital.

Population estimation

Many public policy decisions and community health assessments are based on rates and other characteristics rather than just counts. The calculation of a rate requires an accurate numerator - a subset of the population that meets certain criteria - as well as an accurate denominator, such as the total population in a section of a city or the age- and/or sex-specific count of residents. The total number of residents can be projected from the number of houses in each section and the average household density of a section. The sectional map for Bo can serve as a baseline for estimating the current population of Bo, which is an essential step toward having an accurate "denominator" for the estimation of rates of disease and other characteristics of residents of Bo.

A preliminary examination of population size was conducted in the two sections of Bo located nearest to Mercy Hospital, Kulanda Town and Njai Town. First, remote sensing was used to identify structures. Then, census data from 2004 was used to estimate the population size. Finally, a household survey was conducted to determine the total number of inhabited buildings and the total number of residents in each of the residential structures. These participatory methods could later be implemented in the other sections of Bo identified on the section map. Figure 2 shows the household map for Kulanda Town and Njai Town. There were 316 potential residential structures in Kulanda Town and 260 in Njai Town.

An estimate of population density per household was possible based on the estimated number of buildings shown in remote imagery and data from the 2004 national census data. In 2004, Bo District had a population of approximately 450,000, of which about 150,000 resided in the urban center of Bo [21,22]. The average

Table 1 Sections (neighborhoods) of Bo, sectional areas, and number of Mercy Hospital Laboratory patients

Location	Area (km^2)	Visits	Location	Area (km^2)	Visits
All	30.10	797	New Site North	0.32	4
Kulanda Town *	0.30	274	Fargoya	0.54	3
Samami+	0.59	55	CKC area+	0.46	3
Bo No. 2+	0.48	55	Hangha	0.09	3
Bo No. 2 Extension	1.08		Yemoh Town	0.40	3
Njai Town+	0.21	47	Kebbie Town	0.28	3
Kissi Town+	0.20	36	Salina	0.47	2
Sewa Raod area	0.64	33	Fulawahun	0.08	2
Njaboima	1.75	31	Kindia Town	0.15	2
New London	0.60	28	Clerks' Quarter+	0.08	2
Manjama	0.74	23	Komende	0.20	1
Manjama Extension	0.70		Dodo	0.05	1
Kennedy	0.64	19	Goma	0.27	1
New York	1.51	17	Bo Commercial+	0.18	1
Simbo Town+	0.14	16	Bo Airfield	0.43	1
Nduvuibu+	0.49	15	Tengbewabu	0.68	1
Messima	0.49	15	Old Police Barracks	0.23	1
Bo Central	0.07	13	Toubu	0.02	1
Korwama	0.30	11	New Site South	0.69	1
Kandeh Town	0.54	9	Bunumbu	0.18	1
Kandeh Town Extension	1.32		Brima Town	0.19	0
Kortubguma	0.52	9	VO Vaama	0.15	0
Dorba Ground	0.63	9	Kortumahun	0.35	0
Lewabu	0.48	8	Eastern Police Barracks	0.23	0
Moriba Town	0.25	6	Kpetewoma	0.20	0
Torkpoi Town	0.55	6	Nikibu	0.10	0
Bumpewulo	0.15	6	Kpandobu	0.03	0
Gbondo Town 1	0.72	5	Mulema Town	0.43	0
Gbondo Town 2	0.31		Roma	0.04	0
Reservation	2.33	5	Borbor Kombor	1.22	0
Goba Town+	0.21	5	Moibawo Farm	0.50	0
Gbanja Town	0.64		Chinese Farm	0.10	0
Gbonda Town+	0.12	4	Clock tower area	0.11	0
Joe Town 1	0.12		Mendewa	0.47	0
Joe Town 2+	0.42				

Note: The second column shows the areas of each section of Bo; the third column shows the number of people from each section who were tested at the Mercy Hospital Laboratory. (* denotes the section in which Mercy Hospital is located and + denotes sections with at least half their area within 1 km of Mercy Hospital. Some adjacent sections are grouped together for visit analysis.)

number of residents per household ranged from 6.0 to 7.0 within the various census tracts within Bo [22]. If each building is home to one household and each has an intermediate household density of about 6.5 residents, then about 3700 individuals (2000 in Kulanda Town and 1700 in Njai Town) were estimated to reside in the two sections. However, this estimate was suspected to be inaccurate both because it is difficult to distinguish rooftops of residences from rooftops buildings used for other purposes and because of expected population growth between 2004 and 2010.

To obtain a better estimate of the current population density within houses in Bo, we conducted a census of Kulanda Town and Njai Town. As an initial step, the number of actual residential structures was determined to be 197 in Kulanda Town and 130 in Njai Town, which was less than the number of building rooftops identified from the remote imaging. The average

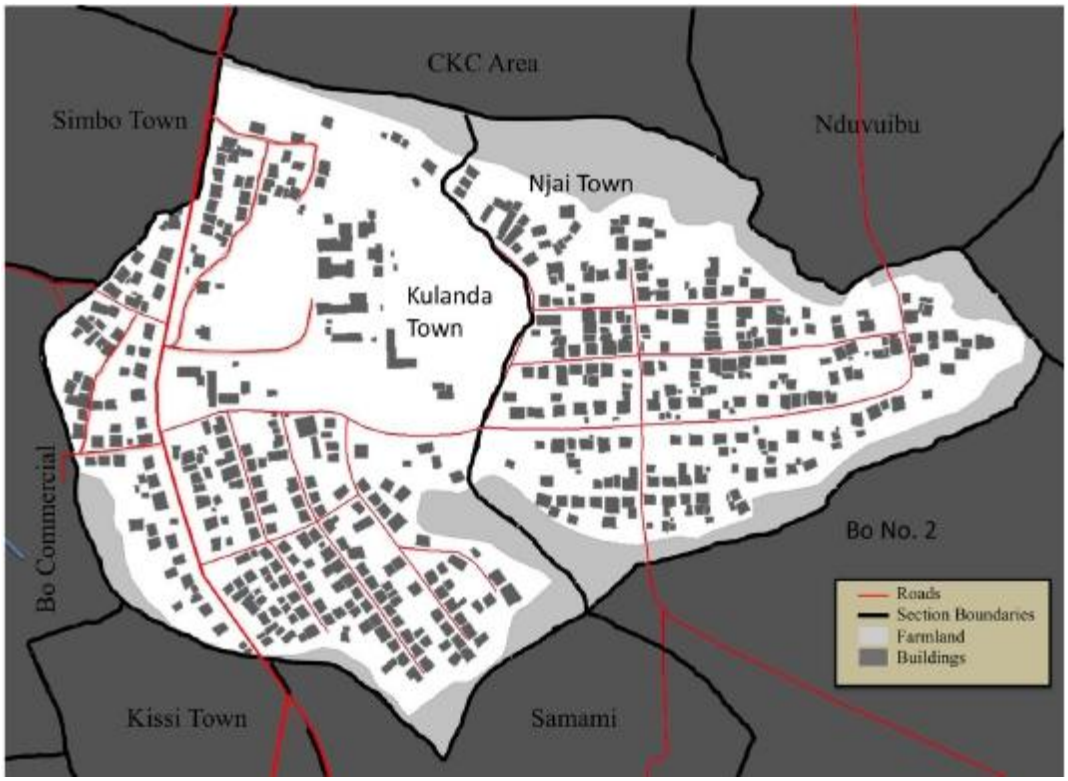

Figure 2 Building structures in Kulanda Town and Njai Town. Results of the Kulanda Town and Njai Town rooftop extraction (dark blue) with roads (red lines), section boundaries (black lines), and farmland (green areas). The dark grey boxes are the results of digitization of building rooftops from images.

household population size was 6.1 people. This was within the range of estimates from the 2004 national census. However, we found that many houses that would typically be classified as modest single-family residences were home to multiple semi-independent households (often composed of relatives who maintain separate sleeping and cooking areas). The 327 houses were home to 1027 households (637 in Kulanda Town and 390 in Njai Town) with a total of 6245 residents, for an average of 19.0 people per building (19.7 for Kulanda Town and 17.9 for Njai Town). The total number of residents in Kulanda Town is then not the 2000 estimated from the map and 2004 census data, but 3894, a striking difference.

Based on the new population estimate for the section, we estimate that 7.0% of Kulanda Town residents were tested at the Mercy Hospital Laboratory during the study period. Having a more accurate denominator for the rate significantly reduces the estimate of the proportion of residents of Kulanda Town who visited the laboratory for testing during the study period from 13.7% to 7.0%. For Njai Town, the total resident population was 2351, and only 2.4% of Njai Town residents were tested at Mercy Hospital Laboratory during the study period. This more accurate number is essential for health services planning. In this example, the lower proportion of the population being served by Mercy Hospital may provide evidence that the local population is underserved by health service providers. Additional survey work in other sections of Bo with varying numbers of residential structures and expected population densities will be required to better understand how to infer population size and density from remote sensing, and how to better define hospital catchment areas and utilization of health care resources.

Conclusions

Participatory methods made possible the creation of detailed, accurate maps for Bo, Sierra Leone, and determined the population of two sections despite a very limited budget for the project. The importance of maps for public policy and other applications was highlighted by two simple demonstrations, the identification of a catchment area for one hospital and the use of the new sectional map to identify houses eligible to participate in a community census. This analysis showed that the majority of the hospital's patients reside within the area adjacent to the hospital and that the number of patients

from a section decreases as the distance from the hospital increases. Some sections outside this area had non-negligible numbers of visits but determining the significance of this is not possible at this time since this study showed that population counts from the 2004 census were not accurate estimates of the current population. Those sections might have a high population density, which would mean that the rate of attendance at Mercy Hospital was not elevated compared to other sections equally distant from the hospital.

It is interesting to note that although we determined that the average population per household was similar to that found in the 2004 census, the average house was home to 3.1 households. Multiple households within one house could be caused by several factors alone or in combination: unavailability of housing in a rapidly urbanizing area, lack of affordability of housing due to demand that raises prices, or cultural factors that value living in close proximity to relatives and extending hospitality to family members in need. Further interaction with community informants could provide insight into the mechanisms at work in Bo.

The methods developed in this paper build on previous PGIS and PPGIS papers [15-20] and serve as a model for the involvement of communities in the generation of municipal maps and their application to social and health concerns. One of the key observations during field work is that community involvement is often the only way to acquire accurate information about boundaries in areas where residential areas often begin as informal settlements. This study demonstrated effective participatory methods to determine the population of a section and to identify geographic information about the section. Together these will improve the application of spatial epidemiology in low- and middle-income countries by allowing for the calculation of more accurate rates. This paper demonstrates that a participatory mapping approach can mitigate some of the challenges inherent to mapping in low-resource areas and can be a first step toward implementing GIS for public applications, such as disease surveillance, the determination of the preferred location of public services, and the management of future outbreaks.

Methods

Sectional mapping

As a first step toward generating a sectional map, we interviewed knowledgeable long-term local residents and, in consultation with municipal authorities in the Bo City Council and local officials of the MLCPE, sketched out the boundaries of each section. We then georeferenced these boundaries using GPSMAP 60Cx Hiking GPS receivers (Garmin International, Inc., Olathe, KS), to the extent that it was possible to do so given that some of the terrain involved physical obstacles such as swamps and that in some places trespassing laws did not allow us to access boundary areas. Main roads were also tracked. The raw GPS tracks were loaded into DNR Garmin software (MapSource-trip and waypoint manager), converted to KML format for intermediate processing. The KML file structure allows coordinates to be adjusted using a text editor and the results of the updates could be confirmed by viewing in Google Earth before final import into ArcMap.

We asked local elders who had resided within the section for at least 15 years to review the map and check our preliminary map boundaries for accuracy. We also received input from Bo city officials. Based on the feedback of both the elders and the city officials, we made further adjustments to the KML description of each section, including adjustments to coordinates so that all locations within the town fell into one defined section. A final review of the boundaries was then made by all participating parties. After our local experts had approved the boundaries, we used DNR Garmin software (longitude and latitude coordinates having UTM WGS84 Zone 29 reference data) to convert the section boundaries from the KML format into a shapefile. This shapefile was imported into ArcGIS. In ArcCatalog, a new polygon shapefile was created and dragged into the ArcMap content table. ArcMap was then used to create a digital polygon of the Bo municipal sections by tracing the boundary lines using the editor toolbar.

Catchment area study

Patient records from Mercy Hospital and its Laboratory, a private medical facility located on the north side of Bo in the Kulanda Town section (Figure 1), were used for the assessment of catchment area. Mercy Hospital Laboratory maintains electronic records of all patients referred to it for testing. The records contain patient demographic data, such as age, sex, and home address (often recorded as only the section of residence when a street address is not available), as well as the results of the various tests that were conducted at the laboratory. These records were queried for all Mercy Hospital patients who were referred for testing to Mercy Hospital Laboratory between February 2009 and March 2010. When laboratory information about residential location was missing, it was filled in from the patient's outpatient records when available. To protect patient privacy, no personally identifiable information, such as patient names or addresses, was included in our search records, and our analysis in this paper is aggregated at the section level.

Population estimation

The first task required to make an estimation of population in a section is to determine the number of houses

in the section. Because software that automatically marks houses by identifying rooftops from remote imagery is, at present, quite expensive, we were unable to use this specialty software and had to digitize the location of homes manually. In ArcMap a new empty window was opened and given WGS 84 UTM Zone 29 reference data. Using the 'Add data' tool, a georeferenced satellite image was added to provide a background image for digitization. ArcCatalog was then opened and new shapefiles were created to represent buildings and streets/roads and given WGS84 UTM Zone 29 reference data. These shapefiles were then added to the ArcMap. The database table of the polygon shapefile representing buildings was opened and a new field added to contain the map section data within which a building was located. Using the ArcEditor tool, the shapefile of interest was selected and the Sketch Tool was used to create a footprint of a particular feature, using a line to represent streets and a polygon to represent buildings.

Once the buildings in each section were identified, an initial physical pass was made through the streets to note which buildings could clearly be identified as being used for purposes other than a residence, such as barns, sheds, and businesses. A notation was added to the map to indicate viable residences and these were assigned unique identifiers.

Every identified residence was visited and an adult resident asked to identify how many people lived in the household. (The household survey was approved by the research ethics committees of Njala University, Bo; George Mason University, Fairfax, Virginia, USA; and the U.S. Naval Research Laboratory, Washington, DC, USA.) The initial visits revealed that residents would usually report that multiple "households" were present within their homes. For all buildings visited, the query included the number of households living in the building and the number of individuals who were members of each household. Two households in Kulanda Town (99.7% participation rate) and five households in Njai Town (98.7% participation rate) declined to participate. The average household population was determined for each section from the households that participated. The estimated population was calculated as this average multiplied by the total number of households within the section. In a similar manner the total number of individuals per building was calculated only from buildings in which all households participated.

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Authors' contributions

RA was involved in study conception, survey collection, and drafting of the manuscript. APM was involved in the study conception, analysis of data, and drafting the manuscript. ASB was involved data collection and preparation of the sectional mapping and house mapping and contributed to the manuscript. AJS was involved data collection and preparation of the sectional mapping and house mapping. DHJ performed survey and patient information collection. UB performed survey and patient information collection and was involved in study conception. KHJ was involved in the design of the census and revising the manuscript critically for important intellectual content. BL was involved in revising the manuscript critically for important intellectual content. DAS was involved in study conception, manuscript revision and gave final approval of the version to be published. All authors have read and approve of the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Cromley EK: Gis and Disease. Annual Review of Public Health 2003, 24:7-24.
2. Hay SI, Omumbo JA, Craig MH, Snow RW: Earth observation, geographic information systems and Plasmodium falciparum malaria in sub-Saharan Africa. Advances in Parasitology 2000, 47:173-215.
3. Kay BA: THe role of the laboratory in disease surveillance. Eastern Mediterranean Health Journal 1996, 2:4.
4. McLafferty SL: GIS and health care. Annu Rev Public Health 2003, 24:25-42.
5. Dulin MF, Ludden TM, Tapp H, Blackwell J, de Hernandez BU, Smith HA, Furuseth OJ: Using Geographic Information Systems (GIS) to understand a community's primary care needs. J Am Board Fam Med 2010, 23:13-21.
6. Bloom BR, Michuad CM, La Montagne JR, Simonsen L: Priorities for global research and development of interventions. In Disease control priotities in developing countries. 2 edition. Edited by: Jamison DT, Breman J, Measham AR, Alleyne G, Claeson M. Washington D.C.: The World Bank; 2006:103-118.
7. Clements ACA, Pfeiffer DU, Martin V, Pittliglio C, Best N, Thiongane Y: Spatial risk assessment of Rift Valley fever in Senegal. Vector-Borne and Zoonotic Diseases 2007, 7:203-216.
8. Thacker SB, Birkhead GS: Surveillance. In Field epidemiology. 2 edition. Edited by: Gregg MB. Oxford; New York: Oxford University Press; 2002:26-50.
9. Galeon F: Estimation of Population in informal settlement communities using high resolution satellite image. XXI ISPRS Congress, Commission IV. Bejjing 2008, XXXVII(Part B4):1377-1381.
10. Harvey JT: Population estimation models based on individual TM pixels. Photogrammetric Engineering and Remote Sensing 2002, 68:1181-1192.
11. Jensen JR, Cowen DC: Remote sensing of urban suburban infrastructure and socio-economic attributes. Photogrammetric Engineering and Remote Sensing 1999, 65:611-622.
12. Lu DS, Weng QH, Li GY: Residential population estimation using a remote sensing derived impervious surface approach. International Journal of Remote Sensing 2006, 27:3553-3570.
13. Schneider A, Woodcock CE: Compact, dispersed, fragmented, extensive? A comparison of urban growth in twenty-five global cities using remotely sensed data, pattern metrics and census information. Urban Studies 2008, 45:659-692.
14. Viel JF, Tran A: Estimating Denominators Satellite-Based Population Estimates at a Fine Spatial Resolution in a European Urban Area. Epidemiology 2009, 20:214-222.

15. Sieber R: Public participation geographic information systems: A literature review and framework. Annals of the Association of American Geographers 2006, 96:491-507.
16. Rambaldi G, Kyem PAK, McCall M, Weiner D: Participatory Spatial Information Management and Communications in Developing Countries. The Electronic Journal on Information Systems in Developing Countries 2006, 25:1-9.
17. Dunn CE: Participatory GIS - a people's GIS? Progress in Human Geography 2007, 31:616-637.
18. Dongus S, Nyika D, Kannady K, Mtasiwa D, Mshinda H, Fillinger U, Drescher AW, Tanner M, Castro MC, Killeen GF: Participatory mapping of target areas to enable operational larval source management to suppress malaria vector mosquitoes in Dar es Salaam, Tanzania. Int J Health Geogr 2007, 6:37.
19. Mamam S, Lane T, Ntogwisangu J, Modiba P, vanRooyen H, Timbe A, Visrutaratna S, Fritz K: Using Participatory Mapping to Inform a Community-Randomized Trial of HIV Counseling and Testing. Field Methods 2009, 21:368-387.
20. Jankowski P: Towards Participatory Geographic Information Systems for community-based environmental decision making. J Environ Manage 2009, 90:1966-1971.
21. Final Results 2004 Population and housing census. Edited by: Leone SS. FreeTown: Statisitcs Sierra Leone; 2004.
22. Koroma DS, Turay AB, Moigua MB: Analytical Report on Population projection for Sierra Leone.Edited by: Leone SS. FreeTown: Statisitcs Sierra Leone; 2006:.

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